Loss of AngiomiR-126 and 130a in Angiogenic Early Outgrowth Cells from Patients with Chronic Heart Failure: Role for Impaired in vivo Neovascularization and Cardiac Repair Capacity

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Abstract: BACKGROUND: MicroRNAs are key regulators of angiogenic processes. Administration of angiogenic early outgrowth cells (EOCs) or CD34(+)-cells has been suggested to improve cardiac function after ischemic injury in particular by promoting neovascularization. The present study therefore examines regulation of angiomiRs, microRNAs involved in angiogenesis, in angiogenic-EOCs and circulating CD34(+)-cells from patients with chronic heart failure (CHF) and the role for their cardiac repair capacity. METHODS AND RESULTS: Angiogenic-EOCs and CD34(+)-cells were isolated from patients with CHF due to ischemic cardiomyopathy (n=45) and healthy subjects (HS; n=35). In flow-cytometry analyses angiogenic-EOCs were largely myeloid and positive for alternatively-activated, M2-macrophage markers. In vivo cardiac neovascularization and functional repair capacity were examined after transplantation into nude mice with myocardial infarction (MI). Cardiac transplantation of angiogenic-EOCs from HS markedly increased neovascularization and improved cardiac function, whereas no such effect was observed after transplantation of angiogenic-EOCs from patients with CHF. RT-PCR analysis of 14 candidate angiomiRs, expressed in angiogenic-EOCs, revealed a pronounced loss of angiomiR-126 and -130a in angiogenic-EOCs from patients with CHF, that was also observed in circulating CD34(+)-cells. Anti-miR-126 transfection markedly impaired the capacity of angiogenic-EOCs from HS to improve cardiac function. miR-126-mimic transfection increased the capacity of angiogenic-EOCs from patients with CHF to improve cardiac neovascularization and function. CONCLUSIONS: The present study reveals a loss of angiomiR-126 and -130a in angiogenic-EOCs and circulating CD34(+)-cells from patients with CHF. Reduced miR-126 expression was identified as a novel mechanism limiting their capacity to improve cardiac neovascularization and function that can be targeted by miR-126-mimic-transfection.

DOI: https://doi.org/10.1161/CIRCULATIONAHA.112.093906

Wolfrum, Mathias; Noll, Georg; Ruschitzka, Frank; Lüscher, Thomas F; Landmesser, Ulf (2012). Loss of AngiomiR-126 and 130a in Angiogenic Early Outgrowth Cells from Patients with Chronic Heart Failure: Role for Impaired in vivo Neovascularization and Cardiac Repair Capacity. Circulation, 126(25):2962-2975.
DOI: https://doi.org/10.1161/CIRCULATIONAHA.112.093906

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Circulation. published online November 7, 2012;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539

DOI: 10.1161/CIRCULATIONAHA.112.093906

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Running title: *Jakob et al.; AngiomiRs, Angiogenic EOCs and Cardiac Repair Capacity*

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Journal Subject Codes: [129] Angiogenesis; [151] Ischemic biology - basic studies

DOI: 10.1161/CIRCULATIONAHA.112.093906

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Methods and Results—Angiogenic-EOCs and $CD34^+$-cells were isolated from patients with CHF due to ischemic cardiomyopathy (n=45) and healthy subjects (HS; n=35). In flow-cytometry analyses angiogenic-EOCs were largely myeloid and positive for alternatively-activated, M2-macrophage markers. *In vivo* cardiac neovascularization and functional repair capacity were examined after transplantation into nude mice with myocardial infarction (MI). Cardiac transplantation of angiogenic-EOCs from HS markedly increased neovascularization and improved cardiac function, whereas no such effect was observed after transplantation of angiogenic-EOCs from patients with CHF. RT-PCR analysis of 14 candidate angiomiRs, expressed in angiogenic-EOCs, revealed a pronounced loss of angiomiR-126 and -130a in angiogenic-EOCs from patients with CHF, that was also observed in circulating $CD34^+$-cells. Anti-miR-126 transfection markedly impaired the capacity of angiogenic-EOCs from HS to improve cardiac function. miR-126-mimic transfection increased the capacity of angiogenic-EOCs from patients with CHF to improve cardiac neovascularization and function.

Conclusions—The present study reveals a loss of angiomiR-126 and -130a in angiogenic-EOCs and circulating $CD34^+$-cells from patients with CHF. Reduced miR-126 expression was identified as a novel mechanism limiting their capacity to improve cardiac neovascularization and function that can be targeted by miR-126-mimic-transfection.

Key words: chronic heart failure; endothelial progenitor cells; ischemic cardiomyopathy; microRNA; angiogenic early outgrowth cells

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Introduction

MicroRNAs (miRNAs), small non-coding RNAs regulating gene expression at the post-transcriptional level, have recently been identified as key regulators of angiogenic processes.[1-4] Endothelial silencing of Dicer, an RNase-III-enzyme required for generation of mature miRNAs, revealed a critical role of miRNAs for *in vitro* and *in vivo* angiogenic processes.[5, 6] Furthermore, individual miRNAs, including miR-126, miR-130a, let-7f and the miR-17-92 cluster, have been identified as key positive or negative regulators of angiogenic processes.[5, 7-13]

Accumulating data suggest that bone marrow-derived mononuclear cells are important regulators of cardiac neovascularization.[14-24] In particular, several studies have demonstrated that administration of angiogenic early outgrowth cells (angiogenic EOCs), also known as circulating angiogenic cells, or $CD34^+$-cells stimulate myocardial neovascularization and improve cardiac function after experimental myocardial infarction (MI).[14-16, 18, 21] Moreover, Fazel et al. have reported that in mice with a mutation of the c-kit receptor, leading to an impaired mobilization of bone marrow-derived progenitor cells post-MI, cardiac neovascularization was substantially impaired associated with an augmented LV-dysfunction post-MI, suggesting an important role of bone marrow-derived mononuclear cells for the endogenous cardiac repair response.[19] More recently, two studies have supported the concept that pro-angiogenic effects of bone marrow-derived mononuclear cells or $CD34^+$-cells are crucial for their capacity to improve cardiac function, using either selective elimination of eNOS or anti-VEGF treatment.[23, 24] Of note, in the TOPCARE-AMI study a beneficial effect of administration of angiogenic EOCs on cardiac function was observed in patients with an acute myocardial infarction,[25] whereas no such effect was detected in patients with an ischemic cardiomyopathy.[26]

The present study was therefore designed to characterize regulation of angiomiRs in

angiogenic EOCs and circulating $CD34^+$-cells from patients with CHF due to ischemic cardiomyopathy and to determine their role for the *in vivo* cardiac neovascularization and functional cardiac repair capacity, as examined after transplantation of angiogenic EOCs into nude mice with myocardial infarction.

Methods

An expanded description of methods is available in the online data supplement.

Characteristics of Patients with CHF and Healthy Subjects

Written informed consent was obtained from all patients and healthy subjects, and the study protocol was approved by the local ethics committee. Angiogenic EOCs and $CD34^+$-cells were isolated from venous blood from patients with chronic heart failure (CHF) due to ischemic cardiomyopathy (ICM; n=45) with left ventricular ejection fraction (LVEF) < 40% and age-matched healthy subjects (HS; n=35). HS had no cardiovascular risk factors (according to history, clinical examination, and laboratory tests) or accompanying disorders. In addition, 15 patients with CHF due to dilated cardiomyopathy without significant coronary stenosis and an LVEF < 40% were included to further examine the effects of CHF independent of ischemic heart disease on angiomiR expression of angiogenic EOCs and $CD34^+$-cells. Patient characteristics are shown in **Table 1.**

Isolation and Cultivation of Angiogenic EOCs and $CD34^+$-cells

Angiogenic EOCs were isolated and cultured as described in detail previously[27-29] and in supplemental methods. Angiogenic EOCs were carefully characterized by flow-cytometry analysis, revealing that the majority of these cells are myeloid and express alternatively-activated, M2-macrophage markers (**Supplemental Figure 1**, **Supplemental Tables 1-3**). For

isolation of $CD34^+$-cells, mononuclear cells were magnetically labeled with CD34-beads and separated using a MACS-column and a MACS-separator according to manufacturer`s instructions (Miltenyi-Biotec).

Animals, Myocardial Infarction and Cell Transplantation

Animal experiments were approved by the local committee. Myocardial infarction (MI) was induced by permanent ligation of left-anterior-descending-coronary-artery in male NMRI-nu/nu-mice, as described in detail previously.[27, 30, 31] Ten minutes post-MI, $5x10^5$ human angiogenic EOCs or placebo (i.e. same volume of PBS-buffer) were injected intramyocardially into the infarct border zone at 4 sites using a 30G needle. Cardiac function was examined by cardiac MRI and Millar-catheter hemodynamic measurements. For details see supplemental methods.

Statistical Analysis

For comparison of three or more groups, the Levene test was used to test for variance heterogeneity. If variance differed significantly between the groups the Welch's ANOVA test was used with Dunnett-T3 test as a post-hoc test. One-way ANOVA followed by Tukey-Kramer test was performed for comparisons between groups with homogenous variance. For comparison of two groups, variance heterogeneity was tested with Levene test followed by t-test or unpaired t-test with Welch's correction, depending on equal or unequal variances, respectively. If there was a non-normal distribution Kruskal-Wallis and Mann-Whitney-U-test were used. All data are expressed as mean±SEM. A p-value of < 0.05 was considered statistically significant. Data were analyzed by using SPSS-20.0.

Results

Cardiac Neovascularization and Repair Capacity of Angiogenic EOCs are Substantially

Impaired in Patients with CHF due to ICM

Cardiac transplantation of angiogenic EOCs from HS into nude mice after myocardial infarction improved LVEF and augmented cardiac function ($dPdt_{max}$), as determined by cardiac MRI and cardiac hemodynamic analysis using the Millar catheter (**Figure 1A-C**; **Table 2**). In contrast, no significant effects on these parameters were observed after transplantation of the same number of angiogenic EOCs from patients with CHF due to ICM (**Figure 1A-C**, **Table 2**).

Capillary density (infarct border zone) was significantly increased after transplantation of angiogenic EOCs from HS, but not after transplantation of angiogenic EOCs from patients with CHF due to ICM (**Figure 1D**), suggesting impaired pro-angiogenic effects of angiogenic EOCs from these patients.

In vivo bioluminescence imaging suggested a similar cell survival of angiogenic EOCs from HS or patients with CHF on day 1 and 3 after cardiac transplantation (**Figure 1E**), consistent with the notion that impaired function may contribute to an impaired cardiac repair capacity of angiogenic EOCs from patients with CHF. Similarly, there was no significant difference of the bioluminescence signal of explanted hearts on day 3 after transplantation of angiogenic EOCs from HS or patients with CHF, and no significant bioluminescence signal was detected in other organs (**Supplemental Figure 2C**). Ex vivo experiments indicated a close relation between increasing cell numbers and the bioluminescence signal (**Supplemental Figure 2B**). Confocal microscopy imaging of Dil-labeled angiogenic EOCs detected transplanted angiogenic EOCs in close vicinity to cardiac microvasculature of the infarct border zone (**Supplemental Figure 3**).

These findings suggested a functional impairment of transplanted angiogenic EOCs from patients with CHF with respect to their capacity to promote *in vivo* cardiac neovascularization.

We therefore determined differential expression of angiomiRs, i.e. miRNAs regulating angiogenic processes, using real-time PCR analysis.

Expression of AngiomiRs miR-126 and miR-130a is Substantially Reduced in Angiogenic EOCs from Patients with CHF due to ICM

First we performed a miRNA-array to detect angiomiRs expressed in angiogenic EOCs from HS (**Supplemental Table 4A**). The expression of fourteen miRNAs, that were expressed in angiogenic EOCs and had a potentially important role in angiogenesis, was compared between angiogenic EOCs from HS and patients with CHF due to ICM by using real-time-PCR analysis (**Figure 2**). Notably, there was a marked loss of expression of potentially pro-angiogenic miR-126 and miR-130a in angiogenic EOCs from patients with CHF due to ICM (**Figure 2**). In addition, expression of potentially anti-angiogenic miR-20a from miR 17-92 cluster was increased in angiogenic EOCs from patients with CHF (**Figure 2**). Due to the pronounced loss of miR-126 and miR-130a expression in angiogenic EOCs from patients with CHF we further examined their role for pro-angiogenic effects of angiogenic EOCs and their capacity to improve cardiac function.

Role of miR-126 and miR-130a for Angiogenic Capacity of Angiogenic EOCs *in Vitro*

Angiogenic effects of angiogenic EOCs were first characterized in co-cultures with human aortic endothelial cells. Angiogenic EOCs from HS markedly stimulated tube formation, whereas no such effect was observed using angiogenic EOCs from patients with CHF (**Figure 3A**). Notably, anti-miR-126 and anti-miR-130a, but not scrambled-miR transfection of angiogenic EOCs from HS impaired their stimulating effects on tube formation *in vitro* (**Figure 3B**, for information on the anti-miR-sequences see **Supplemental Table 5**), indicating a role for both miRNAs for pro-angiogenic effects. Conversely, miR-126-mimic or miR-130a-mimic transfection of angiogenic

EOCs from patients with CHF due to ICM markedly enhanced their capacity to promote tube formation *in vitro* as compared to scrambled-miR transfected EOCs (26.1±4.8% vs. 8.8±4.5%; P<0.05 and 26.4±5.2% vs. 8.8±4.5%, P<0.05, n=7-8, for information on the miR-mimic-sequences see **Supplemental Table 6**). We have further examined effects of miR-126-mimic and miR-130a-mimic transfection of angiogenic EOCs from HS on their capacity to promote tube formation *in vitro,* however, no significant effects on the capacity of angiogenic EOCs from HS to promote tube formation were observed (**Supplemental Figure 4**), although there was a trend for miR-126-mimic transfected angiogenic EOCs from HS for an improved pro-angiogenic effect. A possible explanation for this observation, i.e. the more pronounced effects of miR-126-mimic and miR-130a-mimic transfection on pro-angiogenic capacity of angiogenic EOCs from patients with CHF as compared to angiogenic EOCs from HS could be the up-regulation of respective targets of these miRNAs in angiogenic EOCs from patients with CHF (**Figure 3C**).

We further examined the role of potential targets of miR-126 and miR-130a in angiogenic EOCs for their pro-angiogenic effects. SPRED1 has been identified as a critical target for pro-angiogenic effects of miR-126 in endothelial cells.[7, 8] HOXA5, a target of miR-130a, is a negative regulator of angiogenesis in endothelial cells.[11] Notably, both SPRED1 and HOXA5 expression were substantially up-regulated in angiogenic EOCs from patients with CHF due to ICM, both on mRNA and protein levels (**Figure 3C**, for RT-PCR-primer-sequences see **Supplemental Table 7**). Notably, silencing of SPRED1 and HOXA5 in angiogenic EOCs from patients with CHF due to ICM enhanced their pro-angiogenic capacity in the tube formation assay (**Figure 3D-E**), suggesting that up-regulation of these respective miRNA-targets contributes to impaired pro-angiogenic effects of angiogenic EOCs from these patients.

In line with these observations, anti-miR-126, but not scrambled-miR transfection of

angiogenic EOCs from HS impaired their capacity to stimulate microvessel outgrowth of aortic rings (7.5±7.6% vs. 44.9±9.0%; P<0.05. n=6). There was no statistically significant difference with respect to effects of anti-miR-130a or scrambled-miR transfection of angiogenic EOCs from HS on microvessel outgrowth in the aortic ring assay, however, a trend towards an impaired endothelial outgrowth using anti-miR-130a was observed (26.2±10.2% versus 44.9±9.0%, P=n.s., n=6).

Taken together, these *in vitro* analyses suggested a potential role of down-regulation of miR-126 and miR-130a for the impaired pro-angiogenic capacity of angiogenic EOCs from patients with CHF. We therefore next determined effects of anti-miR-126 and anti-miR-130a transfection of angiogenic EOCs from HS as well as miR-126-mimic and miR-130a-mimic transfection of angiogenic EOCs from patients with CHF on their capacity to improve cardiac neovascularization and cardiac function.

Role of miR-126 and miR-130a for the Capacity of Angiogenic EOCs to Improve Neovascularization and Cardiac Function *in Vivo*

First, angiogenic EOCs from HS were transfected with anti-miR-126, anti-miR-130a or scrambled-miR. Notably, anti-miR-126 transfection markedly impaired the capacity of angiogenic EOCs from HS to improve LVEF as assessed by cardiac MRI and to augment cardiac function as determined by hemodynamic analyses ($dPdt_{max}$) as compared to scrambled-miR transfection (**Figure 4A-B**, **Table 3**). Moreover, anti-miR-126 transfection of angiogenic EOCs from HS impaired their capacity to promote cardiac neovascularization as compared to scrambled-miR transfection (**Figure 4C**).

In our *in vivo* bioluminescence imaging experiments no significant difference in cardiac survival of anti-miR-126 transfected or scrambled-miR transfected angiogenic EOCs from HS

was detected on day 1 and 3 after transplantation (**Figure 4D**), supporting the concept of a functional abnormality of anti-miR-126 transfected angiogenic EOCs.

Anti-miR-130a transfected angiogenic EOCs had an impaired capacity to improve cardiac neovascularization (infarct border-zone) or cardiac function as assessed by hemodynamic analyses ($dPdt_{max}$) (**Figure 4B-C**). There was a trend for a reduced effect of anti-miR-130a transfected angiogenic EOCs from HS on cardiac function as examined by cardiac MRI, however, this did not reach statistical significance (**Figure 4A**).

Subsequently, the effect of miR-126-mimic and miR-130a-mimic transfection on the capacity of angiogenic EOCs from patients with CHF to stimulate cardiac neovascularization and to improve cardiac function was examined. miR-126-mimic transfection of angiogenic EOCs from patients with CHF significantly increased their capacity to improve cardiac function, as shown by cardiac MRI and hemodynamic analysis ($dPdt_{max}$), as compared to scrambled-miR transfection (**Figure 5A-B**, **Table 4**). Moreover, miR-126-mimic transfected angiogenic EOCs from patients with CHF significantly improved neovascularization in the infarct border-zone as compared to scrambled-miR transfection (**Figure 5C**). In studies using miR-130a-mimic transfected angiogenic EOCs from patients with CHF no significant changes with respect to their capacity to stimulate cardiac repair and neovascularization were observed as compared to scrambled-miR transfection, although there was a trend towards an improved capacity to stimulate cardiac repair and neovascularization (**Figure 5A-C**). Of note, over-expression of miR-126 and miR-130a significantly increased the respective miRNA-expression levels of transfected angiogenic EOCs (**Supplemental Figure 5**). The flow-cytometry analysis of surface marker expression of angiogenic EOCs did not reveal significant changes after miR-mimic-126/130a or anti-miR-126/130a transfection (**Supplemental Tables 1-3**).

Circulating CD34$^+$-cells from Patients with CHF have Substantially Reduced miR-126 and miR-130a Levels

To examine, whether observed changes in miR-126 and miR-130a expression are also present in freshly isolated circulating CD34$^+$-cells, miR-126 and miR-130a expression were determined in circulating CD34$^+$-cells from patients with CHF due to ICM and HS. Notably, the expression of both miRNAs was substantially reduced in circulating CD34$^+$-cells from patients with CHF due to ICM as compared to CD34$^+$-cells from HS (**Figure 6A**). Moreover, expression of SPRED1 and HOXA5, important respective targets of these miRNAs, were markedly up-regulated in circulating CD34$^+$-cells from patients with CHF due to ICM (**Figure 6A**). These observations were associated with a markedly impaired pro-angiogenic capacity of CD34$^+$-cells from patients with CHF due to ICM (**Figure 6B**).

To further examine whether down-regulation of miR-126 and miR-130a was limited to patients with CHF due to ICM, we also compared expression of miR-126 and miR-130a in angiogenic EOCs and circulating CD34$^+$-cells from patients with CHF due to dilated cardiomyopathy (DCM) and HS. The expression of both miRNAs was substantially reduced in angiogenic EOCs and CD34$^+$-cells from patients with CHF due to DCM (**Supplemental Figure 6A, 7A**). Moreover, we examined EGF-like-domain-7 (EGFL7) expression, the gene encoding miR-126.[8] There was a trend towards a lower EGFL7-mRNA-expression of angiogenic EOCs from patients with CHF due to ICM or DCM as compared to angiogenic EOCs from HS, however this did not reach statistical significance (**Supplemental Figure 8**). Furthermore, up-regulation of important targets of both miRNAs was observed in angiogenic EOCs and CD34$^+$-cells from patients with CHF due to DCM (**Supplemental Figure 6A, 7A**). In addition, the pro-angiogenic capacity of angiogenic EOCs and CD34$^+$-cells from patients with

CHF due to DCM was markedly impaired (**Supplemental Figure 6B, 7B**). Of note, angiogenic EOCs from patients with CHF due to ICM and DCM showed similar expression levels of miR-126 and miR-130a and their respective targets SPRED1 and HOXA5 (**Supplemental Figure 9**).

Discussion

MicroRNAs (miRNAs) have been identified as critical regulators of angiogenic processes. Experimental and clinical studies have suggested that administration of angiogenic EOCs or $CD34^+$-cells can improve cardiac function following ischemic injury that is attributed in particular to their capacity to promote cardiac neovascularization.[23, 24]

In the present study we have observed a substantial down-regulation of angiomiRs miR-126 and miR-130a in angiogenic EOCs and circulating $CD34^+$-cells from patients with CHF, associated with up-regulation of direct downstream targets of these miRNAs. Furthermore, cardiac transplantation of angiogenic EOCs from HS promoted myocardial neovascularization and improved cardiac function, but no such effects were observed after transplantation of angiogenic EOCs from patients with CHF, indicating a markedly impaired cardiac repair capacity of angiogenic EOCs from these patients. Moreover, targeted delivery of anti-miR-126 to angiogenic EOCs from HS impaired their capacity to improve myocardial neovascularization and cardiac function after ischemic injury *in vivo*, whereas miR-126-mimic transfection of angiogenic EOCs from patients with CHF increased their capacity to improve myocardial neovascularization and cardiac function, suggesting a predominant role of a reduced expression of this miRNA for their impaired cardiac repair capacity. These findings provide novel insights into molecular mechanisms regulating angiogenic and cardiac repair capacity of angiogenic EOCs and their profound alterations in patients with CHF that can be targeted using miR-mimic

transfection.

Several studies have reported that administration of angiogenic EOCs or circulating $CD34^+$-cells can enhance myocardial neovascularization and improve cardiac function.[15, 16, 18, 21, 32] Recent experimental studies have suggested that angiogenic effects of transplanted bone marrow-derived or $CD34^+$-cells are critical for their capacity to promote cardiac repair.[23, 24] Yoon et al. have reported that activation of an inducible suicide gene under the control of the endothelial NO synthase, but not the cardiomyocyte promoter alphaMHC in bone marrow-derived mononuclear cells after cardiac transplantation resulted in reduced effects of BMC transplantation on cardiac neovascularization and function post-MI.[23] Wang et al. have observed that improvement in cardiac neovascularization and function after cardiac $CD34^+$- cell transplantation in mice post-MI was prevented by co-treatment with specific anti-VEGF antibodies, but not with anti-$\alpha4\beta1$ antibodies, further suggesting that angiogenic effects, but not myogenesis, was responsible for functional improvements following $CD34^+$-cell transplantation.[24] In addition, impaired mobilization of bone marrow-derived progenitor cells after experimental MI was associated with a substantially impaired cardiac neovascularization and augmented LV-dysfunction,[19] supporting an important role of angiogenic effects of bone marrow-derived progenitor cells for endogenous cardiac repair responses after ischemic injury.

The present study demonstrates that angiogenic EOCs from patients with CHF, in contrast to angiogenic EOCs from HS, have lost the capacity to promote myocardial neovascularization and improve cardiac function after ischemic injury *in vivo*. Our findings further provide novel insights by demonstrating that altered angiomiR expression in angiogenic EOCs from patients with CHF is on the causal pathway leading to their impaired cardiac repair capacity.

Of note, several cell populations have been suggested to promote cardiac neovascularization, including angiogenic EOCs, $CD34^+$-cells, unselected bone marrow mononuclear cells or mesenchymal stem/progenitor cells.[33] In the present study we have used angiogenic EOCs and $CD34^+$-cells, because there are numerous reports suggesting that these cells can promote neovascularization *in vivo*.[14-16, 18, 21, 24] Moreover, autologous angiogenic EOCs have been tested in a clinical trial in patients with ischemic cardiomyopathy,[26] and an impaired effect on cardiac function has been observed,[26] that was different from the effect observed in patients with an acute myocardial infarction using the same cell type.[25] These findings raised the possibility that angiogenic EOCs from patients with ICM may have impaired pro-angiogenic effects. Notably, the present study suggests that altered angiomiR expression of angiogenic EOCs from patients with CHF due to ICM is critical for their impaired capacity to promote cardiac neovascularization and function.

Of note, the concept of how angiogenic EOCs or $CD34^+$-cells may stimulate cardiac neovascularization has changed over the past years, contributing to modifications in classification and nomenclature of these cell populations.[34-36] Whereas initially several cell populations derived after culture of bone-marrow- or circulating blood-derived mononuclear cells were termed endothelial progenitor cells, later studies have suggested that these cell populations may rather act by paracrine mechanisms to stimulate neovascularization or endothelial repair than by differentiation into true endothelial cells, suggesting that the term endothelial progenitor cells was not adequate for most of these cell populations.[34-36] Our own data suggest in fact that angiogenic EOCs are largely myeloid cells expressing markers of alternatively-activated M2-macrophages. Of note, activation of monocytes and distinct macrophage populations (in particular M2-macrophages) has been shown to promote

angiogenesis, in part by releasing angiogenic factors.[37, 38] In addition, culturing of human peripheral blood mononuclear cells (PB-MNCs) with endothelial growth medium has recently been suggested to stimulate the alternatively-activated, M2-like macrophage phenotype and up-regulation of pro-angiogenic genes.[39] Moreover, in line with this concept recent observations have suggested that endothelial cell co-culture promotes differentiation from several hematopoietic progenitors to macrophages and their polarization towards a pro-angiogenic M2-phenotype.[40]

Furthermore, since angiogenic EOCs, as used in the present study, have to undergo an ex vivo culture period we have also examined expression of miR-126 and miR-130a in freshly isolated circulating $CD34^+$-cells in order to exclude that the observation of a markedly reduced expression of these miRNAs would be limited to ex vivo cultured cells. Indeed, miR-126 and miR-130a expression were markedly reduced in freshly isolated $CD34^+$-cells derived from patients with CHF.

The profound down-regulation of angiomiRs miR-126 and miR-130a, as observed in the present study, is likely particularly prominent in circulating $CD34^+$-cells and angiogenic EOCs from patients with CHF, as it was not detected in LV-tissue samples from patients with severe CHF examined in a recent study.[41] Similarly, in a study that determined the miRNA expression profile in LV-tissue samples from non-failing and failing left ventricles due to DCM, no changes in the left ventricular expression of the above miRNAs have been reported.[42] In a recent study, circulating miRNAs were determined in plasma from patients with CHF, and no significant changes were reported for miR-126 plasma levels.[43]

miR-126 has been suggested to represent an endothelial-lineage specific miRNA and regulates developmental neovascularization.[7-9] Notably, miR-126 has been reported as the most

highly enriched miRNA in embryonic-body derived Flk1-positive vascular progenitor cells.[7] Indeed, one may speculate that the common expression of miR-126 in endothelial cells and hematopoietic progenitor cells may relate to the observation that both hematopoietic and endothelial cells derive from a common precursor, i.e. the hemangioblast, reflecting the common early origin of hematopoietic and endothelial lineages.[44, 45] Experimental studies in miR-126$^{-/-}$ mice have suggested a critical role of miR-126 for cardiac neovascularization and survival post-MI.[8] The present study importantly extents these observations by suggesting a critical role of miR-126 for the cardiac neovascularization and repair capacity of angiogenic EOCs that is dysregulated in patients with CHF. Both studies support the concept that a reduced miR-126 expression may critically impair the cardiac repair response. Less is known about the role of miR-130a in angiogenesis; however, a recent *in vitro* study has suggested that miR-130a exerts important pro-angiogenic effects in endothelial cells, at least in part by inhibiting HOXA5.[11]

Notably, both miR-126 and miR-130a have been suggested to promote angiogenesis by directly repressing important inhibitors of angiogenic processes, including Sprouty-related EVH-1 domain containing-1 (SPRED1) inhibited by miR-126 and HOXA5 repressed by miR-130a in endothelial cells.[7, 8, 11] SPRED1 has been reported to suppress ERK activation and pro-angiogenic signaling.[7, 46] HOXA5 has been described to suppress angiogenic processes by several mechanisms, including down-regulation of pro-angiogenic proteins such as VEGFR2, Ephrin A1 and Hif1alpha, and by up-regulating anti-angiogenic genes such as Thrombospondin-2 (TSP-2).[47] In the present study, both SPRED1 and HOXA5 were up-regulated in angiogenic EOCs and circulating CD34^{+}-cells from patients with CHF, further suggesting a functional relevance of the reduced angiomiR levels. Our SPRED1 and HOXA5-silencing experiments in angiogenic EOCs from patients with CHF demonstrated an improved pro-angiogenic capacity

after specific siRNA-knockdown, suggesting that these intracellular miRNA-targets are important regulators of the pro-angiogenic effects of angiogenic EOCs. The exact mechanisms whereby angiogenic EOCs and $CD34^+$-cells mediate their pro-angiogenic effects are not completely understood, however, paracrine mechanisms are likely involved, including release of exosomes containing several proteins, mRNAs and miRNAs.[21, 48] Of note, SPRED1 has been shown to inhibit activation of ERK/mitogen-activated protein (MAP) kinase,[7, 46, 49] that are also known regulators of VEGF.[50] It is therefore conceivable that these intracellular miRNA-targets inhibit signaling pathways that regulate the paracrine activity of these cells. The exact underlying molecular signaling mechanisms, however, will have to be characterized in future studies.

Furthermore, a recent study has suggested a dysregulation of miR-21 in angiogenic EOCs from patients with coronary artery disease,[51] limiting their migratory capacity. These observations support, together with the present findings, the potential dynamic regulation of miRNAs in angiogenic EOCs in cardiovascular pathophysiological conditions, and may also provide an explanation of why we did not completely restore cardiac repair capacity of angiogenic EOCs using miR-126-mimic treatment.

Moreover, whereas both, anti-miR-126 and anti-miR-130a transfection of EOCs from HS reduced their capacity to promote cardiac neovascularization and improve cardiac function in vivo, only miR-126-mimic transfection significantly improved *in vivo* cardiac repair capacity of EOCs from patients with CHF. Both, miR-126-mimic and miR-130a-mimic transfection improved the pro-angiogenic effects of EOCs from patients with CHF *in vitro*, however, *in vivo* only miR-126-mimic transfection resulted in a significant improvement of their capacity to stimulate myocardial neovascularization and cardiac repair.

This observation raises the possibility that other mechanisms in addition to promotion of direct pro-angiogenic effects of angiogenic EOCs from patients with CHF after miR-126-mimic treatment may contribute to the observed *in vivo* effects. Of note in this respect, a recent study has suggested that miR-126-release can increase SDF-1 expression and promote recruitment of endogenous progenitor cells to sites of injury in atherosclerotic mouse models.[52] Therefore, it is conceivable that miR-126-mimic transfection may exert additional effects on angiogenic EOC-function on top of direct pro-angiogenic properties.

Limitations of the present study

In the present study we cannot exclude that there are also effects of the medication used by patients with CHF on the miRNA expression levels of angiogenic EOCs and $CD34^+$-cells. With respect to statin therapy, miR-126 and miR-130a expression levels of angiogenic EOCs from patients with CHF due to ICM did not differ significantly from angiogenic EOCs from patients with CHF due to DCM, although these patients were largely not on statin therapy. However, future studies will need to characterize effects of different medications on miRNA levels in angiogenic EOCs and $CD34^+$-cells.

In conclusion, the present study demonstrates a substantial loss of angiomiRs miR-126 and miR-130a in angiogenic EOCs and circulating $CD34^+$-cells from patients with CHF, and provides novel insights into their role for the impaired cardiac repair capacity of angiogenic EOCs from patients with CHF. Anti-miR-126 transfection of angiogenic EOCs from HS impairs their capacity to improve myocardial neovascularization and cardiac function. miR-126-mimic transfection improves the capacity of angiogenic EOCs from patients with CHF to stimulate myocardial neovascularization and improves cardiac repair, suggesting a predominant role for this miRNA in regulating cardiac repair capacity of angiogenic EOCs.

Acknowledgments: We thank Simone Kaufmann and Ines Buehler for their excellent contributions to this study.

Funding Sources : This work was supported by Swiss National Research Foundation grants (310030-122339, 33CM30-124112/1), the German Research Foundation (DFG-LA-1432/3-1), the Swiss Heart Foundation, Uniscientia Foundation, the Zurich Center for Integrative Human Physiology and the Clinical Research Focus Program of the University of Zurich.

Conflict of Interest Disclosures: None.

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DOI: 10.1161/CIRCULATIONAHA.112.093906

Table 1. Characteristics of patients

	Healthy subjects	Patients with CHF (ICM)	Patients with CHF (DCM)
n	35	45	15
Age (years)	56.7 ± 1.1	58.6 ± 1.0	57.9±2.4
Gender (m / f)	27/8	38 / 7	12/3
LV-Ejection fraction (%)	62.3 ± 2.7	31.4 ± 1.3 ‡	30.2±3.5 ‡
LV-ESD (cm)	2.7±0.1	5.4±0.2 ‡	5.5±0.3 ‡
LV-EDD (cm)	4.7±0.1	6.6±0.2 ‡	6.4±0.2‡
NT-pro BNP (ng/l)	45 ± 6.0	2018 ± 533 ‡	1577±550 ‡
LDL (mmol/l)	3.4 ±0.1	2.3 ±0.1‡	3.3 ±0.2
HDL (mmol/l)	1.7 ±0.1	1.1 ±0.1 ‡	1.2 ±0.1 †
Creatinine (µM)	82.1 ± 2.5	113.5 ± 6.8 ‡	108.8±12.2 †
BMI (kg/m^2)	23.7 ± 0.8	28.0 ± 0.7 ‡	28.1±1.2 *
NYHA class II/III/IV	-	33/12/0	14/0/1
Medication			
Beta-Blocker		43/45	15/15
ACE inhibitor / ARBs		43/45	14/15
Diuretics		35/45	15/15
Aldosterone antagonists		20/45	07/15
Statins		44/45	05/15
Aspirin		36/45	07/15
Vitamin K antagonists		10/45	07/15

CHF indicates chronic heart failure; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; LV, left ventricular; ESD, end-systolic diameter; EDD, end-diastolic diameter; NT-pro BNP, N-terminal pro B-type natriuretic peptide; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; NYHA, New York Heart Association; ACE, Angiotensin-converting enzyme; ARBs; Angiotensin receptor blockers. Values are mean ± SEM or number of patients. *, P<0.05 vs. Healthy subjects; †, P<0.01 vs. Healthy subjects; ‡, P<0.001 vs. Healthy subjects.

DOI: 10.1161/CIRCULATIONAHA.112.093906

Table 2. MRI and hemodynamic parameters in Sham or MI operated nude mice after treatment with placebo (PBS), angiogenic EOCs from healthy subjects or patients with CHF due to ICM .

	Sham	MI PBS	MI EOCs Healthy	MI EOCs CHF (ICM)
MRI				
LV Ejection Fraction, %	68.5±1.2	27.9±3.0†	42.0±3.9*	22.1±3.9
LV ESV, µl	15.0±1.9	76.6±8.1†	55.4±7.5	80.8±12.1
LV EDV, µl	44.8±4.7	104.2±7.6†	88.9±8.3	102.2±13.1
Hemodynamic data				
LV dP/dt_{max}, mmHg/s	10412±1635	4738±516†	7401±331*	4915±546
LV dP/dt_{min}, mmHg/s	-9539±1101	-4689±489†	-6322±430*	-4218±420
Heart rate, min^{-1}	478±12	472±13	497±17	447±17

LV indicates left ventricle; ESV, end-systolic volume; EDV, end-diastolic volume; MI, myocardial infarction; EOC, angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy. n=8-16 per group. Values are mean ± SEM. *, P<0.05 vs. MI PBS; †, P<0.001 vs. Sham.

Table 3. MRI and hemodynamic parameters in MI operated nude mice after treatment with angiogenic EOCs from healthy subjects, transfected with scrambled-miR, anti-miR-126 and anti-miR-130a.

	MI Healthy Scr-miR	MI Healthy Anti-miR-126	MI Healthy Anti-miR-130a
MRI			
LV Ejection Fraction, %	44.43±4.9	28.3±2.8*	34.0±2.9
LV ESV, µl	49.5±9.5	82.8±9.8*	57.3±4.6
LV EDV, µl	88.1±14.2	113.4±10.3	86.0±3.8
Hemodynamic data			
dP/dt_{max}, mmHg/s	7743±594	5436±100*	5447±606*
dP/dt_{min}, mmHg/s	-6184±397	-5550±241	-4379±392*
Heart rate, min^{-1}	490±13	432±7*	419±15*

LV indicates left ventricle; ESV, end-systolic volume; EDV, end-diastolic volume; MI, myocardial infarction; EOC, angiogenic early outgrowth cells; miR, microRNA; scr-miR, scrambled-microRNA. n= 7-11 per group. Values are mean ± SEM. *, P<0.05 vs. MI Healthy scr-miR

Table 4. MRI and hemodynamic parameters in MI operated nude mice after treatment with angiogenic EOCs from patients with CHF due to ICM, transfected with scrambled-miR, miR-126-mimic and miR-130a-mimic.

	MI CHF (ICM) Scr-miR	MI CHF (ICM) miR-126-mimic	MI CHF (ICM) miR-130a-mimic
MRI			
LV Ejection Fraction, %	20.3±2.3	33.4±1.5*	29.1±6.4
LV ESV, µl	91.0±9.8	52.9±4.3*	76.0±17.3
LV EDV, µl	112.8±10.4	79.1±5.9	102.6±15.8
Hemodynamic data			
dP/dt_{max}, mmHg/s	5029±346	6605±355*	5717±547
dP/dt_{min}, mmHg/s	-4435±362	-5676±411	-5053±1105
Heart rate, min^{-1}	489±9.9	478±12.4	482±5.7

LV indicates left ventricle; ESV, end-systolic volume; EDV, end-diastolic volume; MI, myocardial infarction; angiogenic EOC, early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; miR, microRNA; scr-miR, scrambled-microRNA. n=5-8 per group. Values are mean ± SEM. *, P<0.05 vs. MI CHF (ICM) scr-miR.

Figure Legends:

Figure 1. A, Left ventricular ejection fraction (LV-EF), end-systolic volume (LV-ESV) and end-diastolic volume (LV-EDV), measured by MRI, of nude mice post-MI at day 14 after injection of PBS or angiogenic EOCs from HS and patients with CHF due to ICM. n=8-16 per group. B, Representative short-axis views of a mid-ventricular slice for end-diastolic volumes from MRI measurements. Scale bar=200 um. C, LV-dPdtmax, measured with Millar catheter, of nude mice 14 days post-MI and injection of PBS or angiogenic EOCs from HS and patients with CHF due to ICM. D, Capillary density (border zone between scar and remote myocardium) of mice after injection of PBS or angiogenic EOCs from healthy subjects and patients with CHF due to ICM post-MI at day 14. Above the graph photographs of capillary density are shown, stained for CD31 (brown); arrows are pointing to capillaries. E, Bioluminescence imaging of cell survival

on day 1 and 3 after transplantation of angiogenic EOCs from healthy subjects and patients with CHF due to ICM in the infarct border zone. Background bioluminescence signal is denoted by the red line. Representative bioluminescence images are shown. n=5-6 for each time point. MI indicates myocardial infarction; EOCs, angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy.

Figure 2. Expression of miRNAs involved in angiogenesis in EOCs from patients with CHF due to ICM and HS. n=8. EOCs indicate angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; miR, microRNA.

Figure 3. A, Effect of angiogenic EOCs from HS and patients with CHF due to ICM on HAEC-mediated tube formation. On the right panel photographs of tube formation are shown. n=7-8. B, Effect of transfection with scrambled-miR, anti-miR-126 and anti-miR-130a on *in vitro* pro-angiogenic effects of angiogenic EOCs from HS, as detected by tube formation. On the right panel photographs of tube formation are shown. n=4-6. C, SPRED1 expression – target of miR-126 – and HOXA5 expression – target of miR-130a - on mRNA and protein level in angiogenic EOCs from HS and patients with CHF due to ICM. n=4-8. D and E, Effects on HAECs mediated tube formation after transfection of angiogenic EOCs from patients with CHF due to ICM with scrambled siRNA (scr-siRNA), siRNA for SPRED1 (siRNA-SPRED1) or HOXA5 (siRNA-HOXA5). n=7-17. EOCs indicate angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; miR, microRNA. HAECs, human aortic endothelial cells.

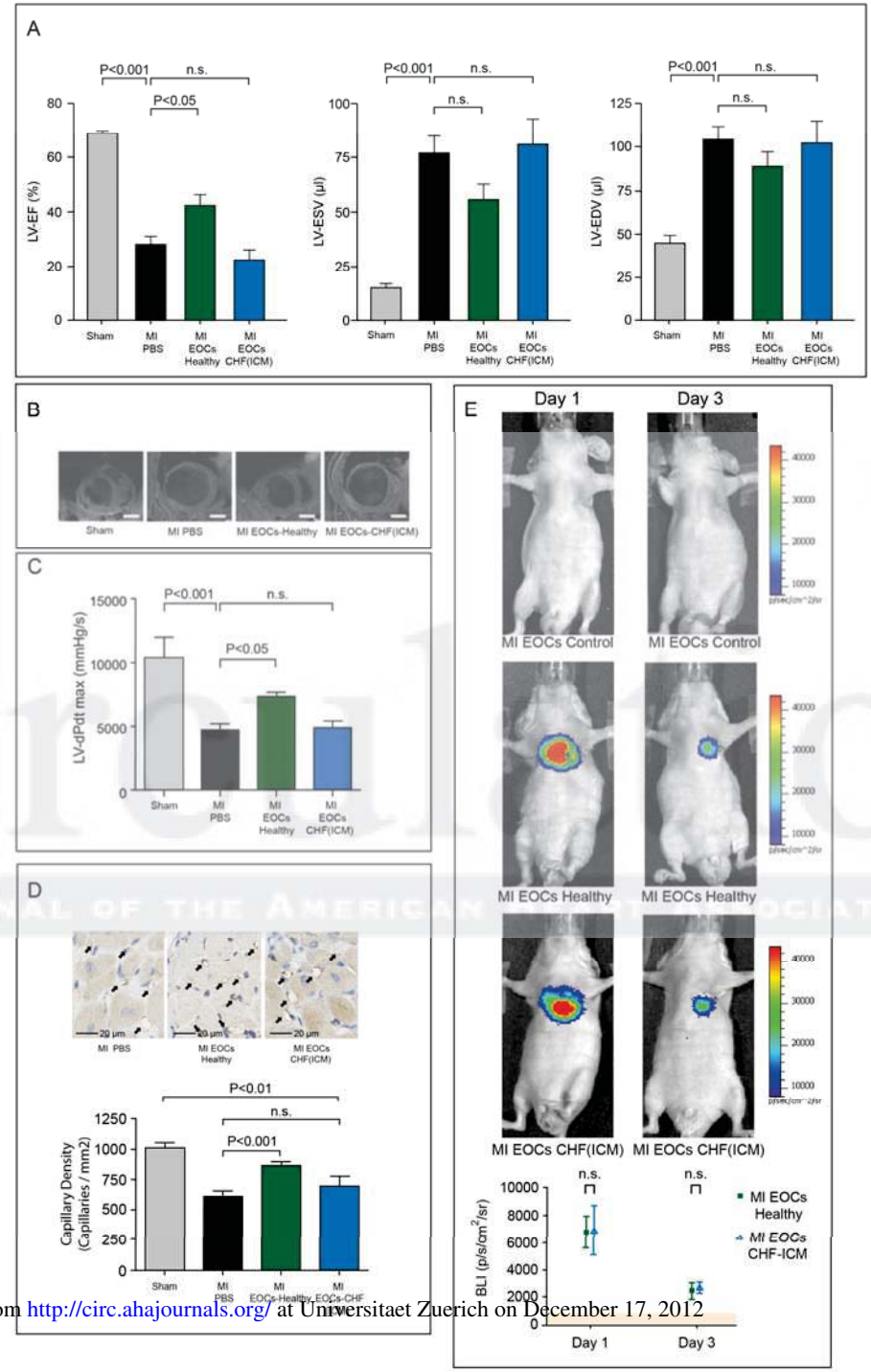
Figure 4. A, Left ventricular ejection fraction (LV-EF), end-systolic volume (LV-ESV) and end-

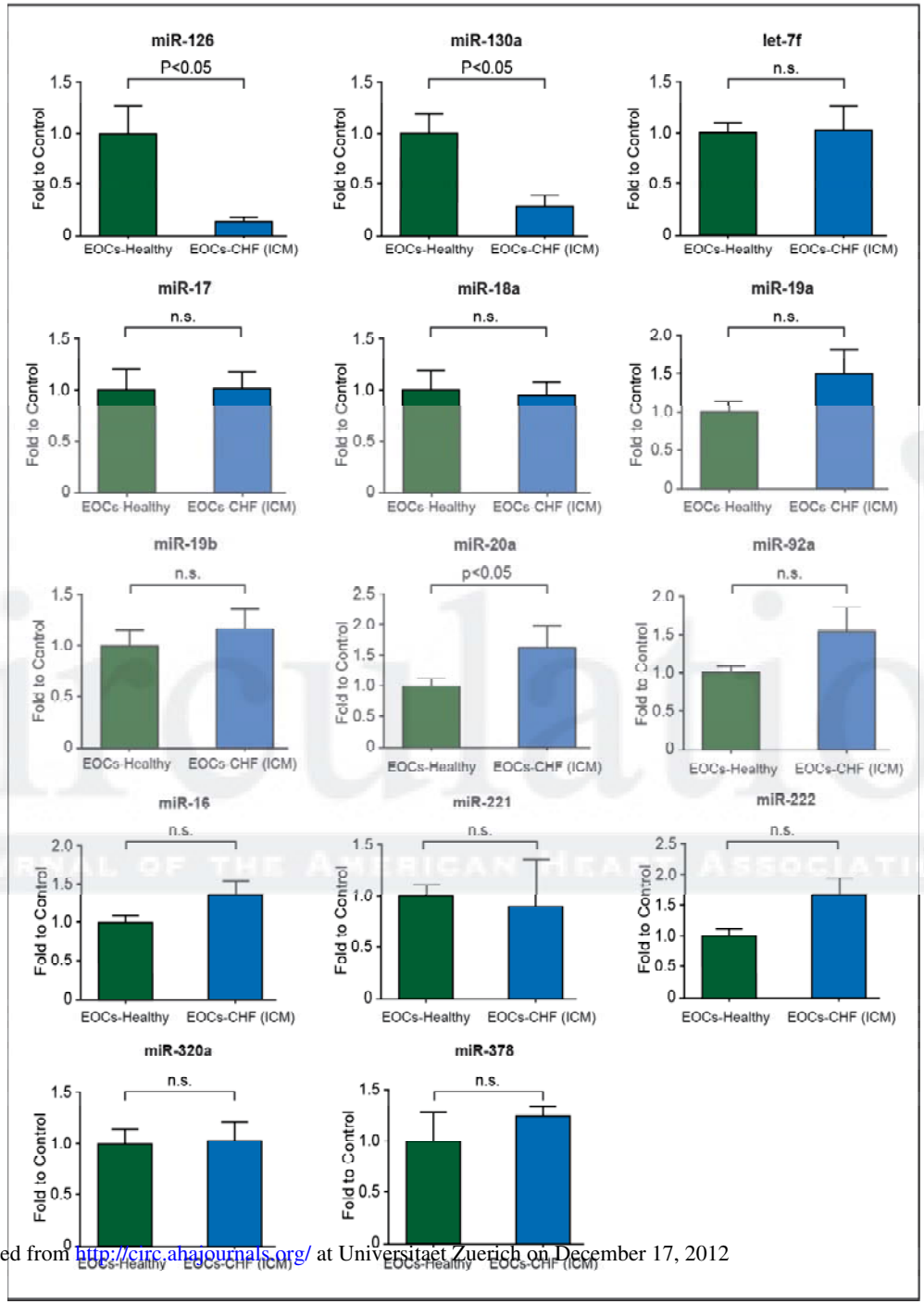
diastolic volume (LV-EDV), measured by MRI, of nude mice post-MI at day 14 after injection of PBS or angiogenic EOCs from HS transfected with scrambled-miR, anti-miR-126 and anti-miR-130a. n=7-11 per group. B, LV-dPdtmax, measured with Millar catheter, of nude mice after injection of angiogenic EOCs from HS transfected with scrambled-miR, anti-miR-126 and anti-miR-130a post-MI at day 14. C, Capillary density (border zone between scar and remote myocardium) of mice after injection of angiogenic EOCs from HS transfected with scrambled-miR, anti-miR-126 and anti-miR-130a post-MI at day 14. Above bar graphs: photographs of capillary density, stained for CD31 (brown), the arrows are pointing to capillaries. D, Bioluminescence imaging of cell survival on day 1 and 3 after transplantation of angiogenic EOCs from HS transfected with scrambled-miR and anti-miR-126. Background bioluminescence signal is denoted by the red line. n=5. EOCs indicate angiogenic early outgrowth cells; MI, myocardial infarction; miR, microRNA.

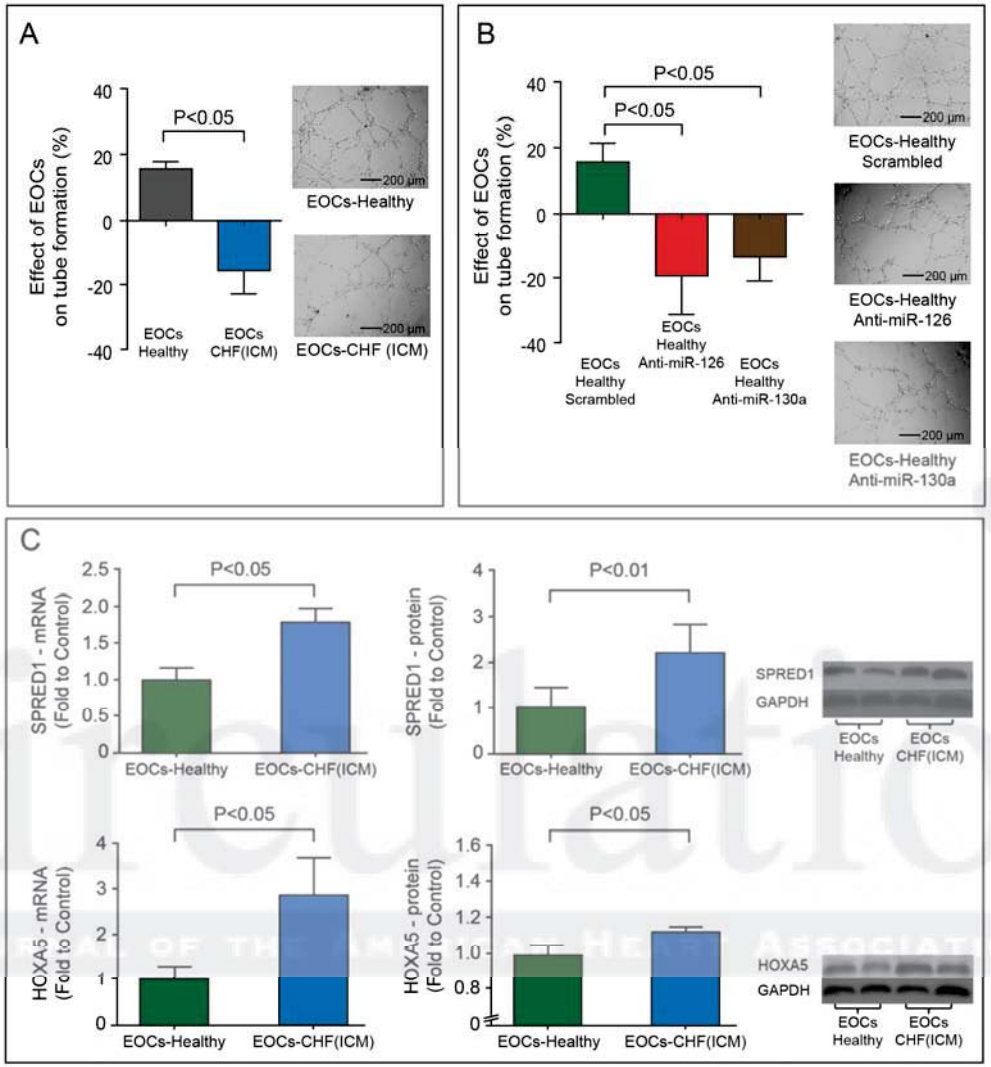
Figure 5. A, Left ventricular ejection fraction (LV-EF), end-systolic volume (LV-ESV) and end-diastolic volume (LV-EDV), measured by MRI, of nude mice after transplantation of angiogenic EOCs from patients with CHF due to ICM transfected with scrambled-miR, miR-126-mimic and miR-130a-mimic post myocardial infarction at day 14. n=5-8 per group. B, LV-dPdtmax, measured with Millar catheter, of mice after injection of angiogenic EOCs from patients with CHF due to ICM transfected with scrambled-miR, miR-126-mimic and miR-130a-mimic post-MI at day 14. C, Capillary density (infarct border zone) of mice after injection of angiogenic EOCs from patients with CHF due to ICM transfected with scrambled-miR, miR-126-mimic and miR-130a-mimic post-MI at day 14. Above bar graphs: photographs of capillary density, stained for CD31 (brown), the arrows are pointing to capillaries. EOCs indicate angiogenic early

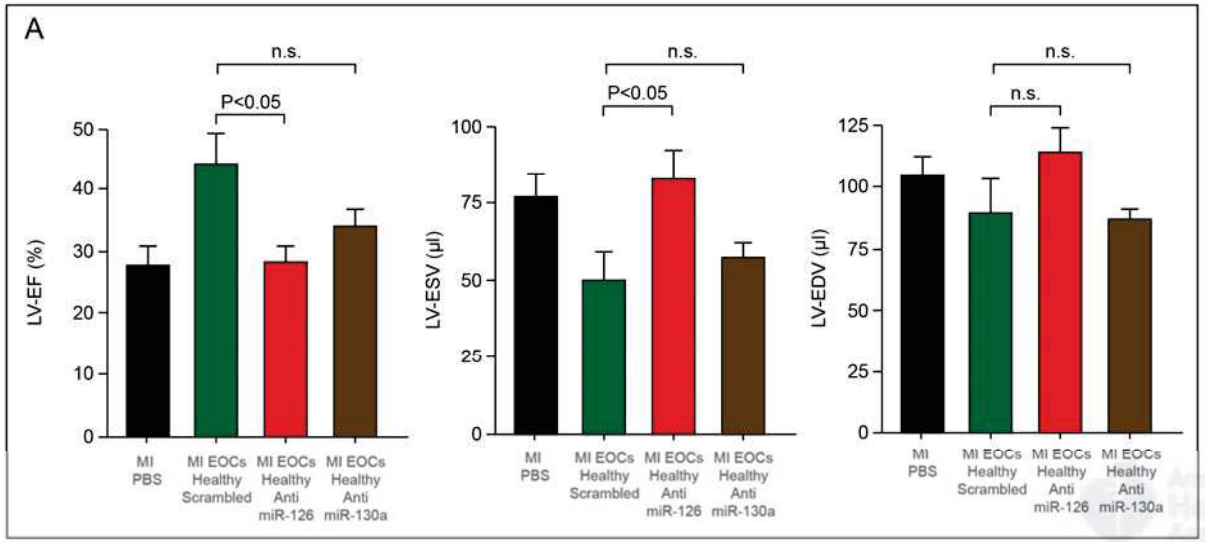
outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; MI, myocardial infarction; miR, microRNA.

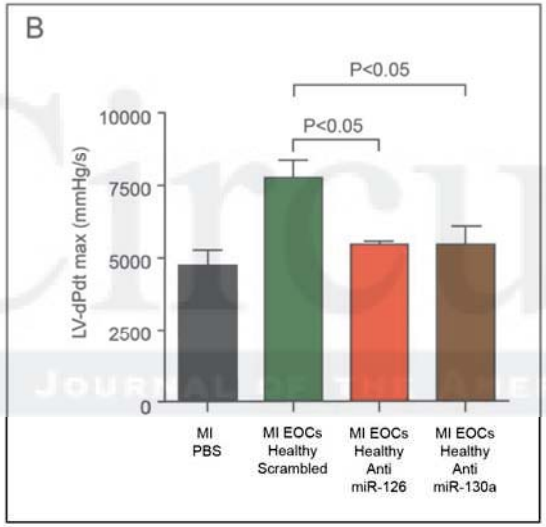
Figure 6. A, Expression of miR-126 and miR-130a and their targets - SPRED1 and HOXA5 - in circulating $CD34^+$-cells from HS and patients with CHF due to ICM. B, Analysis of the effect of $CD34^+$-cells from HS and patients with CHF due to ICM on HAEC-mediated tube formation in matrigel. On the right panel photographs of tube formation are presented. n=4-8. $CD34^+$ indicates circulating $CD34^+$-cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; miR, microRNA; HAECs, human aortic endothelial cells.

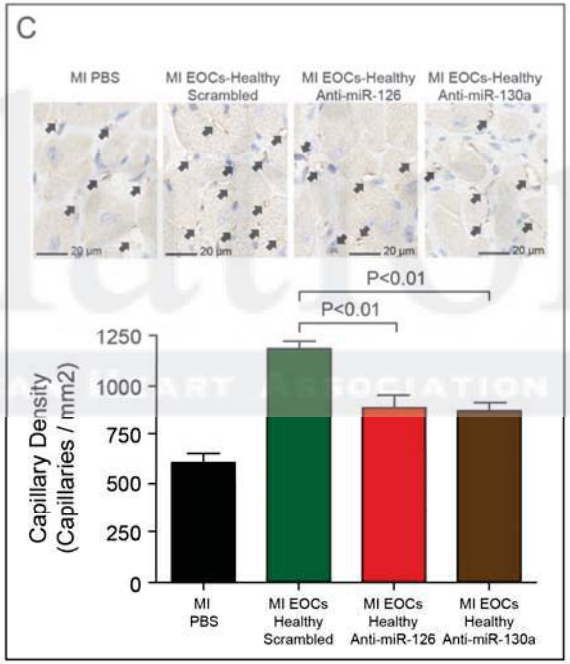


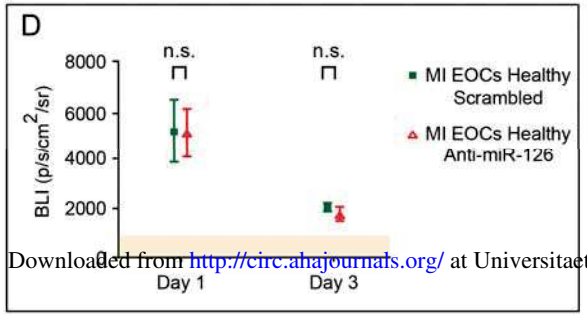


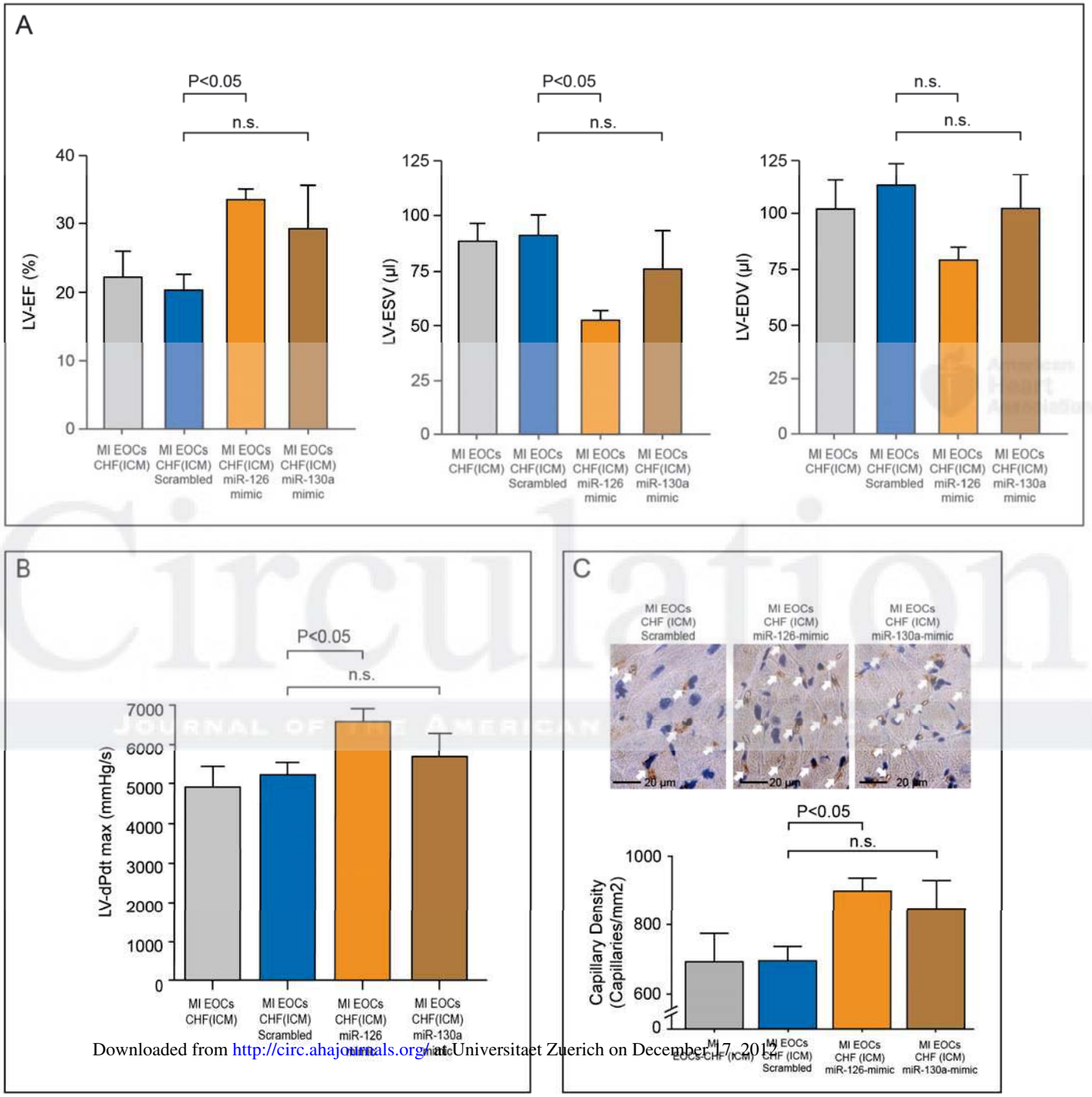


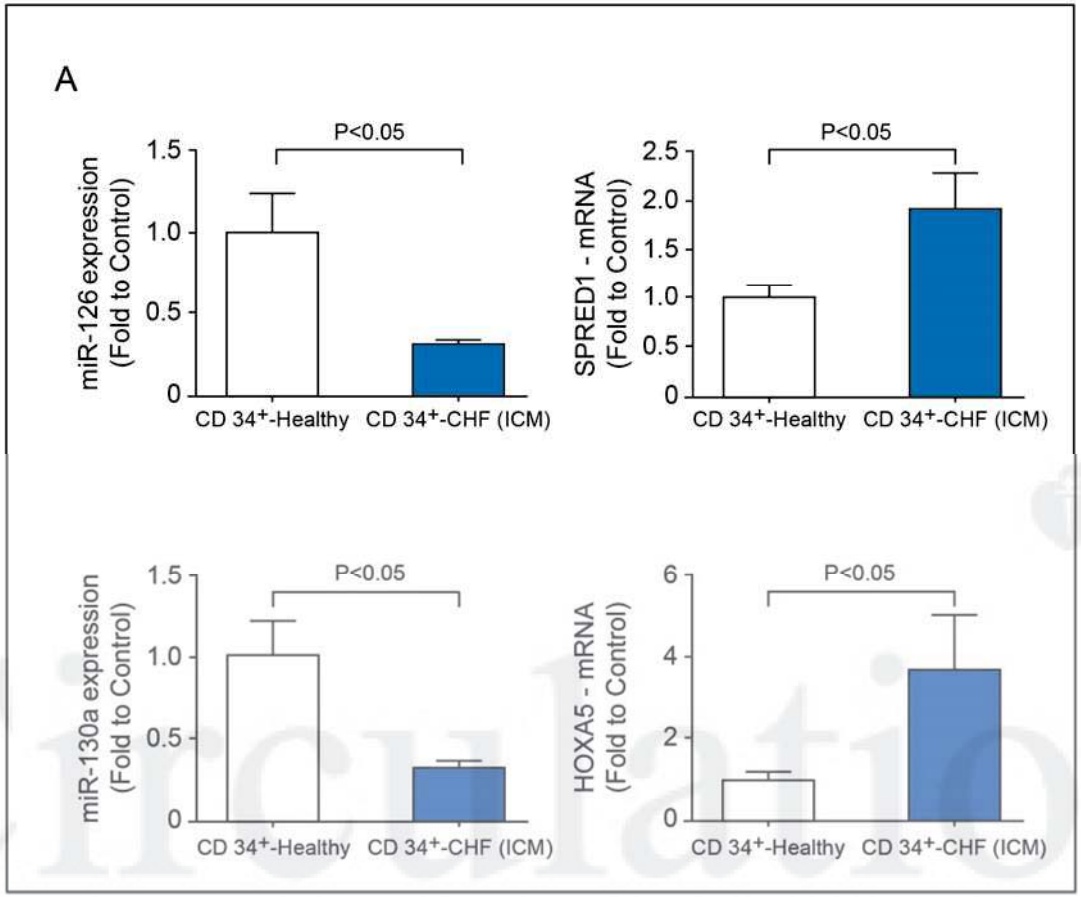


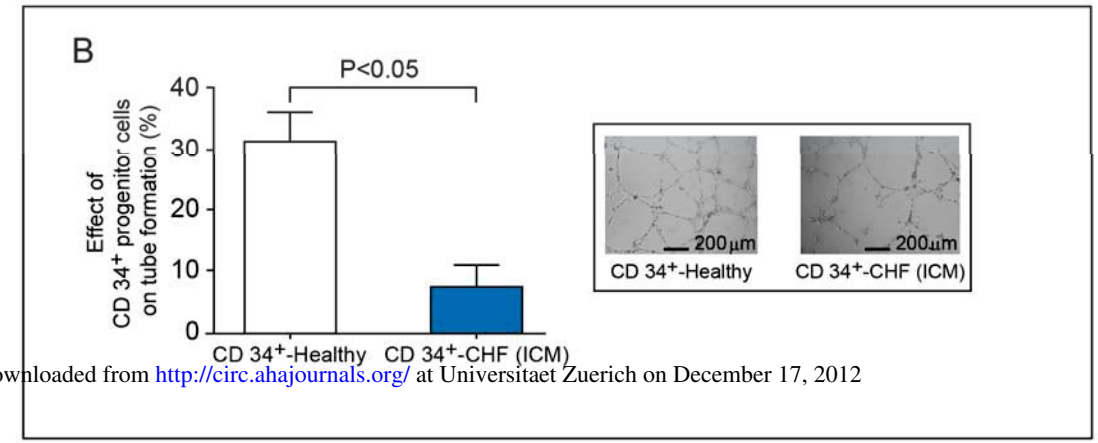












SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Isolation and Cultivation of Angiogenic EOCs and CD34+ Progenitor Cells: Angiogenic EOCs were isolated and cultured as described in detail previously.[1-4] In brief, peripheral blood mononuclear cells were isolated by density gradient centrifugation with LSM 1077 (PAA Laboratories), and $3.8x10^7$ cells were cultured on fibronectin-coated 100 mm dishes in endothelial cell basal medium-2 supplemented with endothelial growth medium. Single quots were used as indicated by the manufacturer, 10 % of fetal bovine serum (FBS) was added. After 4 day culture, nonadherent cells were removed. Remaining cells were trypsinized and used for the *in vivo* and *in vitro* analyses.

Of note, the classification schemes and nomenclature of the different cell populations with pro-angiogenic properties varies in the literature and has been substantially modified by several groups over the past years.[5-7] Whereas initially several different cell populations derived after culture of bone-marrow- or circulating blood-derived mononuclear cells have been termed endothelial progenitor cells, later studies have suggested that most of these cell populations may act to stimulate neovascularization or endothelial repair by paracrine mechanisms rather than by differentiation towards true endothelial cells, suggesting that the term endothelial progenitor cells was not adaequat for most of these cell populations.[7, 8] We have therefore used the term angiogenic early outgrowth cells (EOCs) rather than endothelial progenitor cells in the present study. We have previously reported that these angiogenic EOCs are characterized by cellular uptake of 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine-labeled acetylated LDL (acLDL-DiI) and binding of FITC-labeled Ulex europaeus agglutinin-1 lectin (UEA-1).[1-3, 9] Additionally, angiogenic EOCs from HS and patients with CHF were characterized using flow cytometry (Supplemental Figure 1 and Supplemental Tables 1-3). Angiogenic EOCs from HS were transfected with scrambled-miR, anti-miR-126 or anti-miR-130a and angiogenic EOCs from patients with

CHF were transfected with scrambled-miR, miR-126-mimic and miR-130a-mimic. Thereafter, the cell surface phenotype of angiogenic EOCs was assessed using the following antibodies: CD14, CD86, CD163, CD206, CD3, CD4, CD8, CD19, CD45, CD34, CD133 and KDR (all except CD34 and CD133 (Miltenyi Biotec) from BioLegend) at day 3 after transfection. No changes of cell surface markers were observed after transfection of angiogenic EOCs with miR-126 and miR-130a, indicating that miR-126 and miR-130a expression levels do not affect these cell surface markers (Supplemental Figure 1 and Supplemental Tables 2-3).

Importantly, we have previously clearly demonstrated that angiogenic EOCs, as examined in the present study, can promote endothelial repair after vascular injury in vivo, but the transplanted angiogenic EOCs were located adjacent to the endothelial cell layer rather than becoming endothelial cells, therefore likely acting by paracrine mechanisms.[1, 4]

For isolation of CD 34+ cells, mononuclear cells were magnetically labeled with CD34-beads and separated in a MACS-column placed in a MACS-separator according to the manufacturer`s instructions (Miltenyi-Biotec).

Animals, Myocardial Infarction and Cell Transplantation: Animal experiments were approved by the local committee. Male NMRI nu/nu mice, aged 10-16 weeks were used for transplantation of human angiogenic EOCs. Mice were housed under specific pathogen-reduced conditions receiving autoclaved chow and water ad libitum. Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending coronary artery, as described in detail previously.[2, 10-12] An injection anesthesia (Medetomidin – 20 µg/kg /Climazolam – 0.2 mg/kg /Fentanyl – 2 µg/kg) was used for the operation. Ten minutes post MI, $5x10^5$ human angiogenic EOCs or placebo (i.e. the same volume of PBS buffer) were injected intramyocardially into the infarct border zone at 4 sites using a 30G needle.

Magnetic resonance imaging (MRI), hemodynamic measurements (Millar catheter), histomorphometric analysis and immunohistochemistry were performed 14 days after MI. For *in vivo* bioluminescence imaging, $1x10^6$ human Ad-Luc EOCs, anti-miR-126 transfected Ad-Luc EOCs or Ad-GFP EOCs as control were injected post MI.

Cardiac Magnetic Resonance Imaging: MRI experiments were performed on a 9.4 T small animal MRI system (Bruker BioSpin MRI, Ettlingen, Germany) equipped with a gradient system capable of 400 mT/m in a minimum rise time of 80 µs. Animals were anesthetized using 1 to 2% isoflurane in an oxygen/air (80/20 %) mixture. For MR signal transmission and reception a circular polarized birdcage resonator with an inner diameter of 21 mm was used. The self gated cardiac imaging method IntraGate (ParaVision 5.0, Bruker BioSpin, Ettlingen, Germany) was applied to acquire long-axis, short-axis and four-chamber scout views of the heart which were subsequently used as a basis for planning of the cine FLASH sequence. Contiguous 1-mm slices in short axis orientation covering the entire long axis of the heart were acquired. Remaining sequence parameters were: field of view (FOV) = 2.50 cm x 2.50 cm, matrix size = 256 x 256, flip angle = 10°, echo / repetition time (TE / TR) = 1.812 / 57.682 ms resulting in a total scan time of 24 min and 26 sec. In post processing acquired MR image data was assigned to 10 cardiac phases and the end-expiratory motion state according to the self-gating signal. MRI images were analyzed to determine end-diastolic and end-systolic volumes using BioMap 4.2 A (M. Rausch, Novartis, Basel, Switzerland) and subsequently LV ejection fractions were calculated from the obtained values.

Hemodynamic Measurements of Cardiac Function: Hemodynamic measurements were performed using a 1.4F micromanometer conductance catheter (Millar Instruments) as described previously.[11] In brief, animals were anesthetized with isoflurane (1-2%),

mechanically ventilated and the catheter was inserted in the LV cavity via the right carotid artery. For obtaining and analyzing the hemodynamic data the software PVAN 3.6 (Millar) was used.

Histology: Two weeks post myocardial infarction hearts were arrested in diastole with potassium chloride and left ventricles (LV) were harvested as described previously.[2, 10, 11] LV tissue slices were embedded in paraffin, cut into 4-µm sections and immunohistochemistry for CD31 (rabbit polyclonal CD31 Antibody, Abcam, Cambridge, MA, USA) was performed by using a BondMax immunostaining machine (Leica) as instructed by the manufacturer including a refine DAB-kit to determine capillary density. Capillaries in the infarct border zone of LV were counted in 6 high power fields (400x magnification; 200x200 µm) by an investigator unaware of the treatment group and results were expressed as capillaries per mm^2.

For EOC incorporation, angiogenic EOCs were labeled with vybrant Dil (Invitrogen) according to the manufacturer's protocol, washed three times with growth medium and transplanted into the infarct border zone. After 2 days, endothelial cells were labeled with 75 ul of Fluorescein labeled GSL I – isolectin B_4 (Vector Laboratories) via tail vein injection. Hearts were harvested after 45 min. Frozen tissue sections (5 um) were mounted in Vectashield mounting media with 4-,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) and examined by confocal microscopy (SP5 Leica Microsystem, Germany).

Transfection of Angiogenic EOCs: For viral transfection of angiogenic EOCs, peripheral blood mononuclear cells were isolated and cultured on fibronectin-coated 6-well plates. At day 3, non-adherent cells were removed and adherent cells were transfected (multiplicity of infection 400) with an adenovirus carrying luciferase (Ad-Luc EOCs) or GFP (Ad-GFP

EOCs) (ViraQuest, Inc., IA, USA). After 24 hours, virus containing supernatant was removed and, after washing with PBS, transfected angiogenic EOCs were kept in EGM-2 containing 10% FBS till further use. For transfection efficiency, Ad-GFP EOCs were analyzed by FACS. Transfection efficiency was 81.1±3.7%.

Bioluminescence imaging: Cardiac bioluminescence imaging was performed using the Xenogen IVIS 200 system (Caliper Life Sciences). Mice were anaesthetized with 2% isoflurane. 29 minutes after intraperitoneal injection of luciferin (200 mg/kg), images were acquired for 5 minutes on day 1 and 3 post-MI and transplantation of $1x10^6$ human Ad-Luc EOCs from HS and patients with CHF due to ICM, anti-miR-126 transfected Ad-Luc EOCs from HS or Ad-GFP EOCs (control). Imaging signals were quantified using a fixed region of interest (ROI) in photons/sec/cm^2/sr. In addition, bioluminescence signals of freshly explanted organs (heart, lung, liver and spleen) were determined on day 3 after intra-myocardial transplantation.

RNA Isolation, microRNA Array and Real Time PCR Analysis: Total RNA was extracted from angiogenic EOCs and CD34+ cells using QIAzol Reagent (Qiagen) and miRNeasy Mini Kit (Qiagen) according to the manufacturer's instructions.

For miRNA-profiling the miRCURY™ LNA Array from Exiqon (Version 10.0), a hybridization-based array, was used. After hybridization the microarray slides were stored in ozone free environment (ozone level below 2.0 ppb). The miRCURY™ LNA array microarray slides were scanned using the Agilent G2565BA Microarray Scanner System (Agilent Technologies, Inc., USA) and image analysis was carried out using the ImaGene 7.0 software (BioDiscovery, Inc., USA). The quantified signals were normalized using the global Lowess (LOcally WEighted Scatterplot Smoothing) regression algorithm. In this array, the

expression of each miRNA is measured by hybridization using four probes placed across the slide and each miRNA is coded by green fluorescence (Hy3). A mixture of all samples is used as control or common reference and is coded by red fluorescence (Hy5). After hybridization, a Hy3 signal intensity and Hy5 signal intensity of each of the four probes was determined. The median of the four Hy3/Hy5 ratios was calculated and subsequently log2 transformed. In the Supplementary Table 4A the normalized Hy3/Hy5 ratios (log2 transformed) from all 4 samples (Healthy 1 to 4) and the mean values with the standard deviation of miRNAs, which were found to be expressed in angiogenic EOCs are shown.

AngiomiR expression in EOCs and CD34+ cells from patients with ICM or DCM and HS was compared by using real-time PCR analysis. Firstly c-DNA synthesis was performed with 5 ng of total RNA in a final volume of 10 µl, according to the manufacturer's instruction (miRCURY LNA™ first-strand cDNA kit, Exiqon, Denmark). Real-time PCR was performed with a MX3000P PCR cycler (Stratagene, Amsterdam, Netherlands). All experiments were performed in at least quadruplicates using the miRCURY LNA™ microRNA PCR system (Exiqon, Denmark). Each reaction (25 µl) contained 0.4 µl cDNA, 10 pmol of each primer, 0.25 µl of internal reference dye, and 12.5 µl of SYBR Green master mix. The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 40 cycles with a denaturing phase at 95 °C for 20 s and an annealing phase at 60 °C for 1 min. Data were normalized to results obtained with primers specific for U6.

Anti-miR and miR-mimic Transfection: Angiogenic EOCs were transfected with 25 pmol of scrambled-miR, anti-miR-126 or anti-miR-130a (Exiqon, Denmark) or 25 pmol of scrambled-miR, miR-126-mimic or miR-130a-mimic (Ambion) in a total volume of 75 µl in DMEM. The conditions for electroporation were the following: 200 V, 1 pulse and 10 µs (BTX, ECM 830, MA, USA). Cells were incubated for 12 hours at 37°C and 5% CO_2. The

sequences of the used anti-miRs and miR-mimics are listed in the supplemental Table 2 and 3. Transfection efficiency was tested by electroporation of angiogenic EOCs with FITC-labeled scrambled-miR and was > 80 %.

Tube Formation Assay: To assess the pro-angiogenic activity of EOCs and CD34+ cells, tube formation capacity after co-incubation of $2x10^3$ angiogenic EOCs and $8x10^3$ HAECs was examined in 50 µl of Matrigel (BD, growth factor reduced) in EGM 2 medium in 1 well of a 96 well plate for 6 hours. HAECs alone served as positive control, angiogenic EOCs and CD34+ cells alone were used as negative control. The number of completely formed tubes was counted in 5 high power fields (40x magnification) by an investigator unaware of the treatment group. All results were normalized to the positive control.

Aortic Ring Assay: C57BL/6 mice were euthanized and thoracic aortas were harvested. Fibroadipose tissue was carefully removed. Aortas were cut into 1mm pieces. Aortic rings were embedded in Matrigel (BD, growth factor reduced) and incubated with EBM-2 containing 2% FBS (control), anti-miR-126, anti-miR-130a or scrambled-miR transfected angiogenic EOCs in EBM-2 containing 2% FBS. Endothelial sprouting was analyzed by visual counting at day 6 by an investigator unaware of the treatment group. All results were normalized to the control.

Real Time PCR Analysis of SPRED1 and HOXA5: Conversion of total RNA to cDNA was performed using Moloney murine leukemia virus reverse transcriptase and random hexamer primers (Amersham Biosciences, Piscataway, NJ) in a final volume of 33 µl using 0,4 µg of RNA. Real-time PCR was performed using a MX3000P PCR cycler (Stratagene, Amsterdam, Netherlands). All experiments were performed in at least quadruplicate using the SYBR Green JumpStart kit (Sigma). Each reaction (25 µl) contained 2 µl cDNA, 10 pmol of

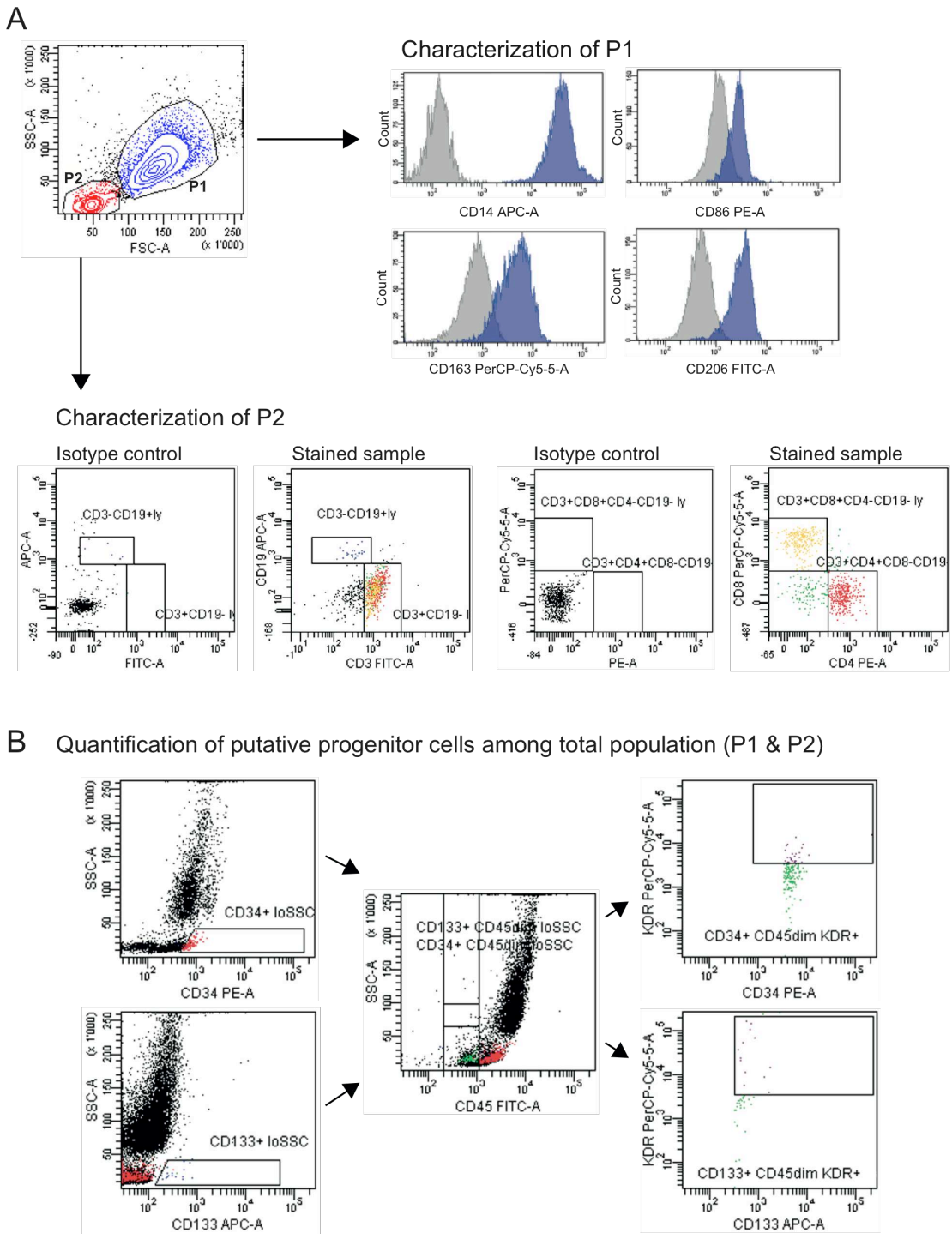
each primer, 0.25 µl of internal reference dye, and 12.5 µl of JumpStart Taq ReadyMix (containing buffer, dNTPs, stabilizers, SYBR Green, Taq polymerase and JumpStart Taq antibody). The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 40 cycles with a denaturing phase at 95 °C for 30 s, an annealing phase at 60 °C for 1 min, and an elongation phase at 72 °C for 1 min. A melting curve analysis was performed after amplification to verify the accuracy of the amplicon, and PCR products were analyzed on an ethidium bromide stained 1% agarose gel. Data were normalized to results obtained with primers specific for human L28, TBP or GAPDH. The sequences of the used primers are listed in the supplemental Table 1.

Western Blot Analysis: Expression of SPRED1 and HOXA5 was analyzed by western blotting as described previously[11], using the following antibodies: SPRED1 antibody (C-term, Abgent; San Diego, CA, USA), HOXA5 antibody (Abcam, Cambridge, MA, USA), a horseradish peroxidase conjugated secondary anti-mouse or an anti-rabbit antibody (Amersham Biosciences). Equal protein loading was verified by reprobing the membrane with a mouse anti-GAPDH antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

siRNA Silencing of SPRED1 and HOXA5: Angiogenic EOCs were harvested for electroporation and $1x10^6$ angiogenic EOCs were transfected with 33 nmol of scrambled siRNA, SPRED1 siRNA (SantaCruz) or HOXA5 siRNA (SantaCruz) in a total volume of 300µl. The conditions for electroporation were the following: 250V, 1 pulse and 125 µFD (BioRad, Gene Pulser). Cells were incubated for 48 hours at 37°C and 5% CO_2, washed and subsequently used to perform *in vitro* tube formation assay.

SUPPLEMENTAL FIGURES

Supplemental Figure 1



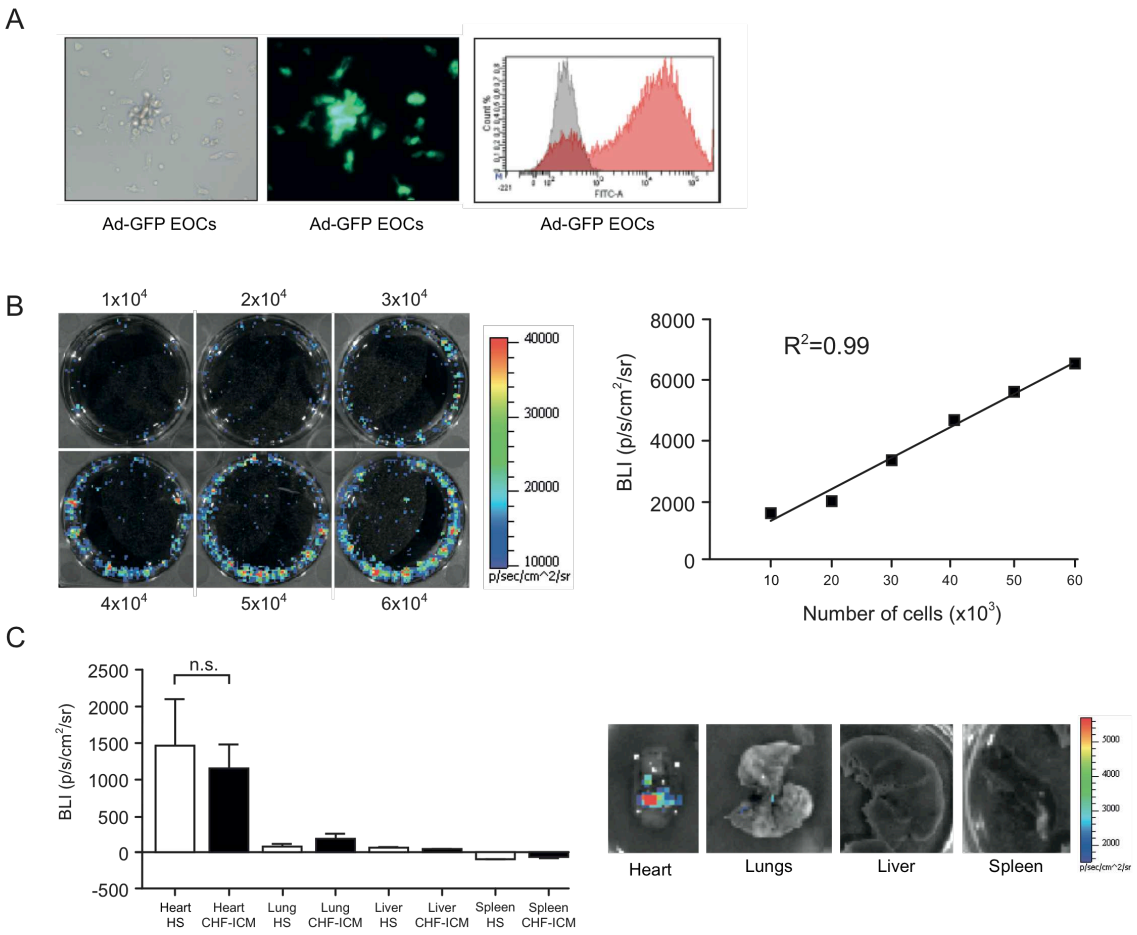
Supplemental Figure 1: A, Characterization of angiogenic EOCs by flow cytometry. Using forward and side-scatter characteristics, a larger population with higher granularity (P1) and a smaller population with low granularity (P2) were identified. Further characterization

revealed that the P1 gate contains largely cells with a myeloid phenotype (positive for CD14 and/or macrophage markers CD86, 163 and 206) and that the P2 gate contained largely cells with a lymphoid marker expression (CD3, CD19). For characterization of P1, isotype control is depicted in grey and a representative stained sample is depicted in blue. For characterization of lymphoid cells in the P2 gate, dot plots were used to first identify CD3-CD19+ B-lymphocytes as well as CD3+CD19- T-lymphocytes (two left panels). CD19-CD3+ lymphocytes were then further characterized with regard to their expression of CD4 or CD8 (two right panels). Isotype control and stained sample are shown as separate plots.

B, Putative progenitor cells within angiogenic EOCs were quantified using a modified ISHAGE-based strategy. Cells with low side scatter and positivity for either CD34 or CD133 were identified (left panel). Among those, cells with low CD45 expression were identified (CD45dim, middle panel). CD34+ CD45dim SSClo as well as CD133+ CD45dim SSClo cells were further characterized with regard to their KDR expression (right panel).

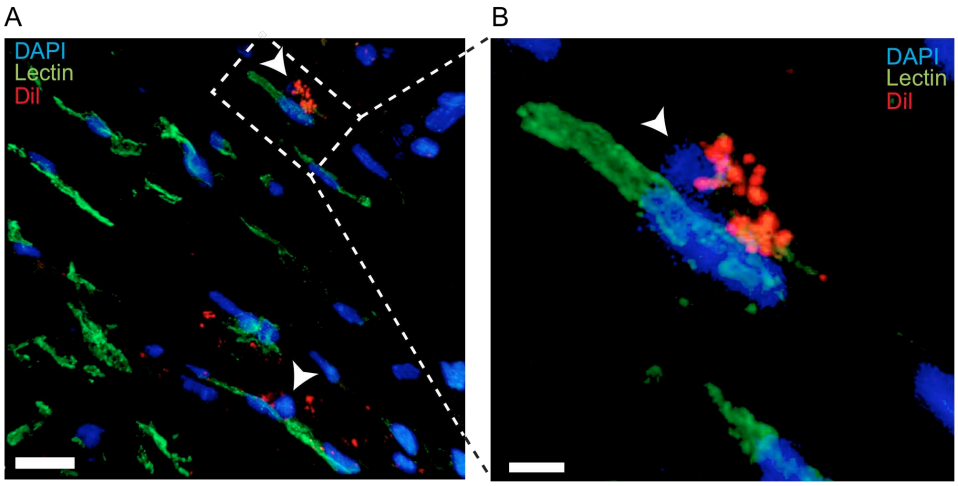
EOCs indicate angiogenic early outgrowth cells; CD, cluster of differentiation; KDR, kinase insert domain protein receptor.

Supplemental Figure 2



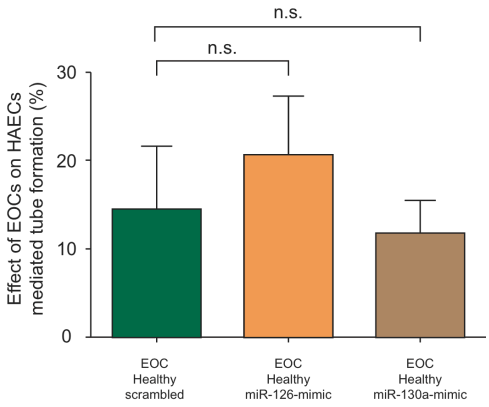
Supplemental Figure 2. Viral transfection of angiogenic EOCs. A, Representative photomicrographs of angiogenic EOCs transfected with an adenovirus carrying GFP (Ad-GFP EOCs), detected by fluorescence microscopy (left panel) and flow cytometric histogram of Ad-GFP EOCs (red filled histogram) (right panel). B, Correlation of cell count of angiogenic EOCs transfected with an adenovirus carrying luciferase and bioluminescence signal *in vitro*. C, Bioluminescence signal of explanted organs on day 3 after myocardial infarction and transplantation of angiogenic EOCs. On the right hand side representative bioluminescence imaging of explanted organs are shown. n=4-5. EOC indicates angiogenic early outgrowth cells; HS, healthy subject; CHF, chronic heart failure; ICM, ischemic cardiomyopathy

Supplemental Figure 3



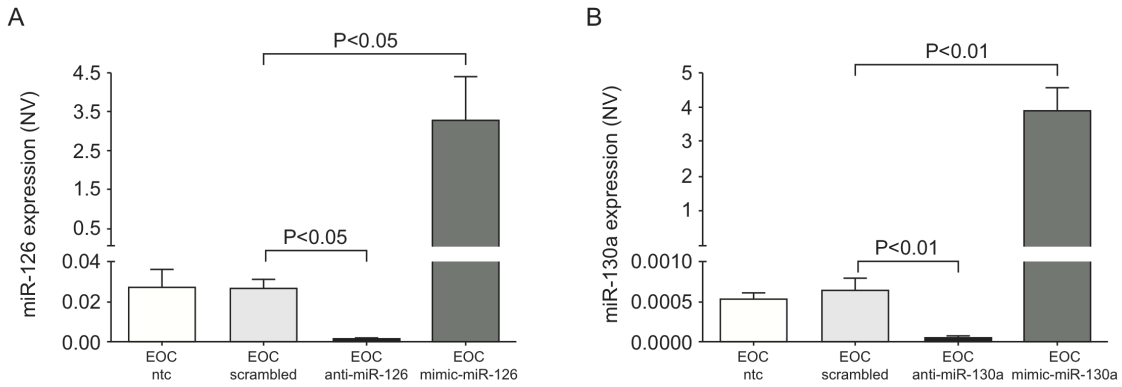
Supplemental Figure 3: Localization and detection of transplanted, vybrant DiI-labeled angiogenic EOCs (red) after myocardial infarction (48h post myocardial infarction). Vessels are stained with Isolectin B4 (green), Nuclei with DAPI (blue). A, Angiogenic EOCs were detected in the infarct border zone; arrows are pointing to the nuclei of angiogenic EOCs. Scale bar=20 um. B, Higher magnification of an EOC detected in close vicinity to an endothelial cell, arrow is pointing to the nucleus of the EOC, scale bar=5um. EOC indicates angiogenic early outgrowth cell; Lectin, Isolectin B4.

Supplemental Figure 4



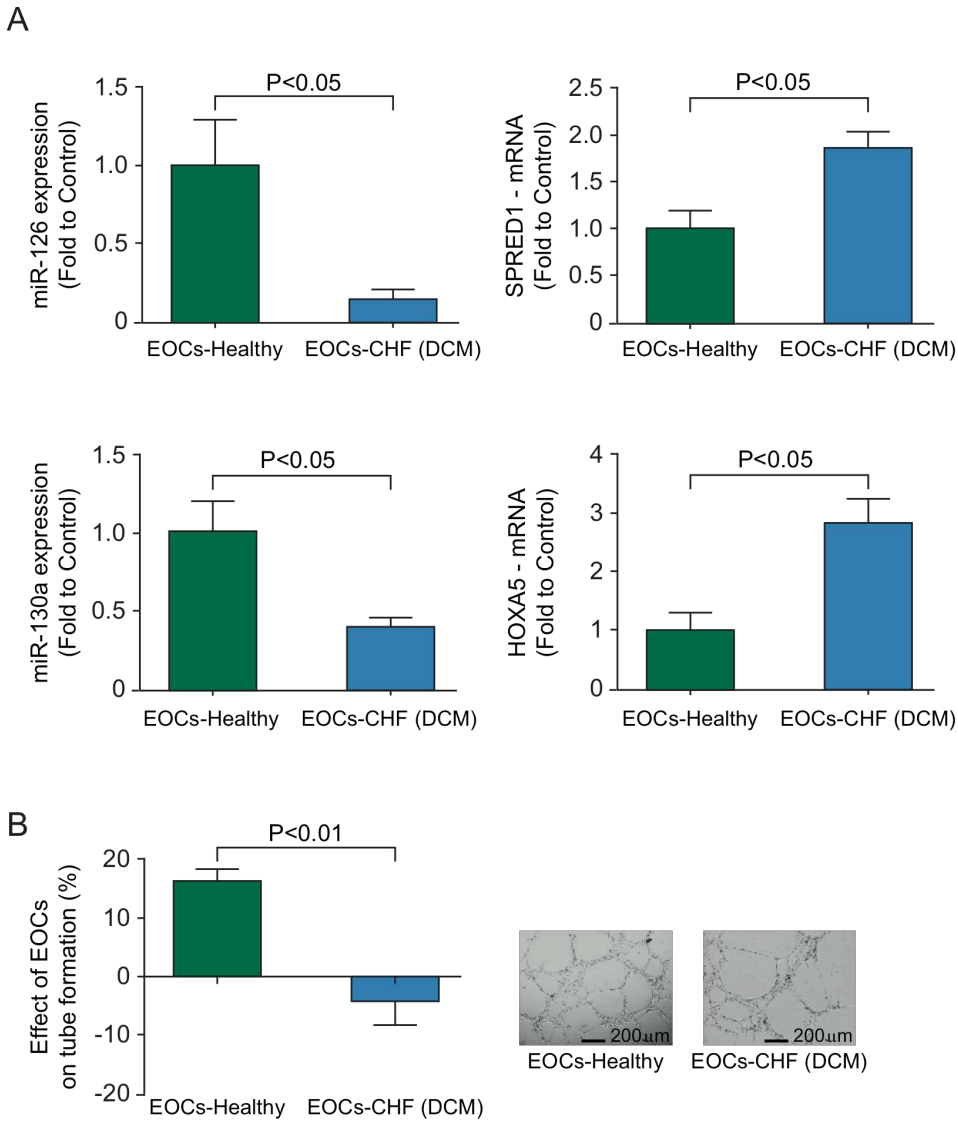
Supplemental Figure 4: Effect of angiogenic EOCs from healthy subjects on HAECs mediated tube formation after transfection with scrambled-miR, miR-126-mimic and miR-130a-mimic. n=8. EOC indicates angiogenic early outgrowth cells; HAECs, human aortic endothelial cells.

Supplemental Figure 5



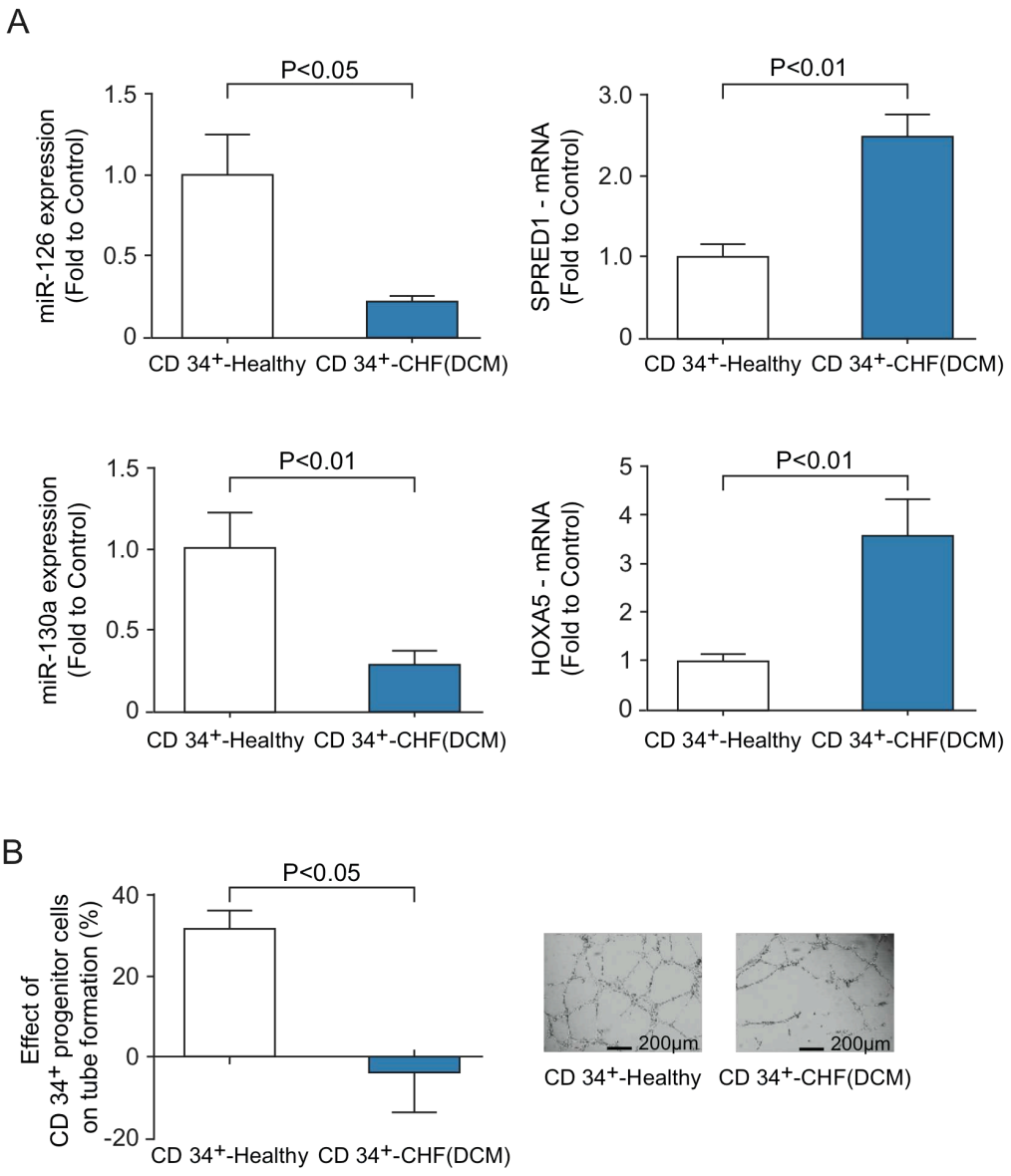
Supplemental Figure 5: miR-126 and miR-130a expression levels in angiogenic EOCs after transfection with (A) miR-scrambled, anti-miR-126 or miR-126-mimic and (B) miR-scrambled, anti-miR-130a or miR-130a-mimic. n=4-12. EOCs indicate angiogenic early outgrowth cells; miR, microRNA.

Supplemental Figure 6



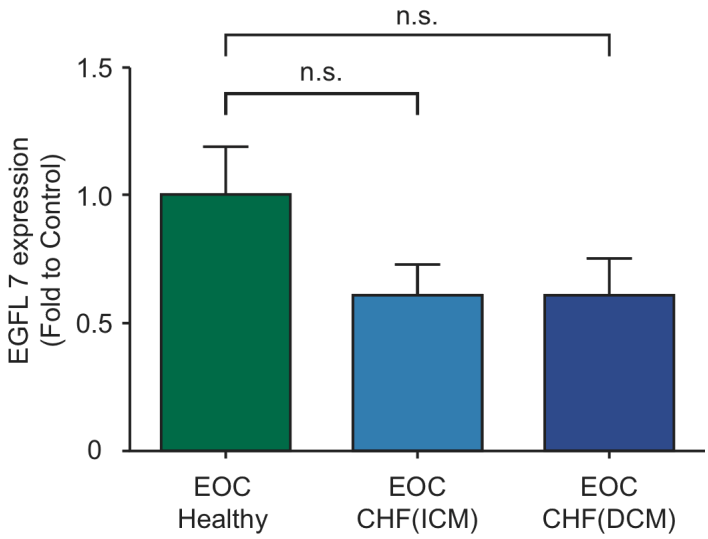
Supplemental Figure 6: A, Expression of miR-126 and miR-130a and their targets - SPRED1 and HOXA5 - in angiogenic EOCs from healthy subjects and patients with CHF due to DCM. n=5-8. B, Analysis of the effect of angiogenic EOCs from healthy subjects and patients with CHF due to DCM on HAECs mediated tube formation in matrigel. On the right panel photographs of tube formation are presented. n=7-8. EOCs indicate angiogenic early outgrowth cells; CHF, chronic heart failure; DCM, dilated cardiomyopathy; miR, microRNA.

Supplemental Figure 7



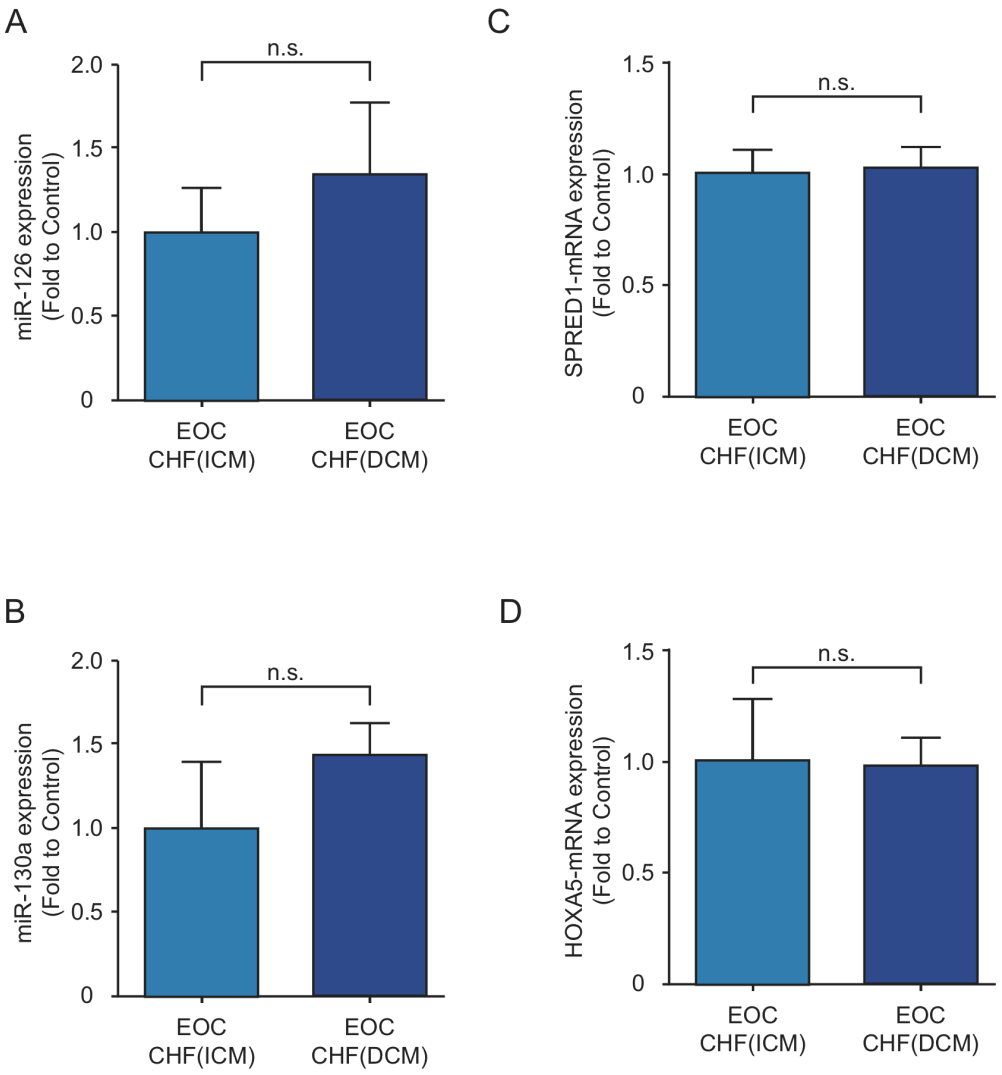
Supplemental Figure 7: A, Expression of miR-126 and miR-130a and their targets - SPRED1 and HOXA5 - in CD34+ cells from healthy subjects and patients with CHF due to DCM. B, Analysis of the effect of CD34+ cells from healthy subjects and patients with CHF due to DCM on HAECs mediated tube formation in matrigel. On the right panel photographs of tube formation are presented. n=4-8. $CD34^+$ indicates circulating $CD34^+$ cells; CHF, chronic heart failure; DCM, dilated cardiomyopathy; miR, microRNA.

Supplemental Figure 8



Supplemental Figure 8: Expression of EGFL7 on mRNA level in angiogenic EOCs from healthy subjects and patients with CHF due to ICM and DCM. n=6-7. EGF-like domain 7 (EGFL7) is the gene encoding miR-126.[13] EOC indicates angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; EGFL7, EGF-like domain 7.

Supplemental Figure 9



Supplemental Figure 9: Comparison of miR-126 and miR-130a and their targets in angiogenic EOCs from patients with CHF due to ICM and angiogenic EOCs from patients with CHF due to DCM. MiR-126 (A) and miR-130a (B) expression levels in angiogenic EOCs from patients with CHF due to ICM as compared to angiogenic EOCs from patients with CHF due to DCM. SPRED1 expression (C) - target of miR-126 - and HOXA5 expression (D) - target of miR-130a - in angiogenic EOCs from patients with CHF due to ICM and DCM. n=5-8. EOC indicates angiogenic early outgrowth cells; CHF, chronic heart failure; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; miR, microRNA.

SUPPLEMENTAL TABLES

Supplemental Table 1. Comparison of cell surface phenotype of angiogenic EOCs from HS and angiogenic EOCs from patients with CHF using flow cytometry

Antigenic marker	EOCs-HS	EOCs-CHF
P1 gate (% positive cells)		
CD14+	90.0±5.8	99.1±0.5
CD86+	58.6±7.0	69.1±6.1
CD163+	76.2±7.4	76.1±3.5
CD206+	82.7±5.4	78.0±5.3
P1&P2 gate (% positive cells):		
CD34+CD45^{dim}	1.1±0.3	0.25±0.1*
CD34+CD45^{dim}KDR+	0.03±0.01	0.01±0.00
CD133+CD45^{dim}	0.09±0.04	0.09±0.06
CD133+CD45^{dim}KDR+	0.06±0.03	0.07±0.05
CD45+	92.7±1.9	94.8±2.3
P2 gate (% positive cells):		
CD3+CD19-	61.3±7.1	65.9±7.1
CD4+CD3+CD8-CD19-	31.6±5.4	29.5±4.8
CD8+CD3+CD4-CD19-	19.2±3.0	28.5±6.9
CD19+CD3-	3.4±1.4	6.1±2.6

HS indicates healthy subjects; CHF, chronic heart failure; EOCs, angiogenic early outgrowth cells (with scrambled-miR transfection). For the gating strategy (P1 and P2 gates) please see supplemental figure 1. n=5-6 per group. Values are mean ± SEM. *, P<0.05 vs. EOCs-HS.

Supplemental Table 2. Comparison of cell surface phenotype of angiogenic EOCs from HS transfected with miR-scrambled, anti-miR-126 or anti-miR-130a using flow cytometry

Antigenic marker	EOCs-HS scrambled-miRNA	EOCs-HS anti-miR-126	EOCs-HS anti-miR-130a
P1 gate (% positive cells)			
CD14+	90.0±5.8	93.3±3.5	90.5±4.2
CD86+	58.6±7.0	59.2±9.0	54.7±6.6
CD163+	76.2±7.4	76.7±6.7	72.0±7.7
CD206+	82.7±5.4	83.2±4.0	78.6±5.8
P1&P2 gate (% positive cells):			
CD34+CD45^{dim}	1.1±0.3	1.1±0.3	1.0±0.3
CD34+CD45^{dim}KDR+	0.03±0.01	0.02±0.01	0.03±0.01
CD133+CD45^{dim}	0.09±0.04	0.07±0.03	0.06±0.02
CD133+CD45^{dim}KDR+	0.06±0.03	0.05±0.02	0.04±0.02
CD45+	92.7±1.9	92.9±1.3	92.9±1.5
P2 gate (% positive cells):			
CD3+CD19-	61.3±7.1	60.1±7.0	60.4±7.4
CD4+CD3+CD8-CD19-	31.6±5.4	31.8±5.3	32.4±5.6
CD8+CD3+CD4-CD19-	19.2±3.0	18.4±3.0	17.8±2.6
CD19+CD3-	3.4±1.4	4.2±1.8	3.8±1.6

EOCs indicate angiogenic early outgrowth cells; HS, healthy subjects; miR, microRNA. For gating strategy (P1 and P2 gates) please refer to supplemental figure 1. n=5-6 per group. Values are mean ± SEM. P = n.s. for all comparisons.

Supplemental Table 3. Comparison of cell surface phenotype of angiogenic EOCs from patients with CHF transfected with miR-scrambled, miR-126-mimic or miR-130a-mimic using flow cytometry

Antigenic marker	EOCs-CHF scrambled-miRNA	EOCs-CHF miR-126-mimic	EOCs-CHF miR-130a-mimic
P1 gate (% positive cells)			
CD14+	99.1±0.5	99.2±0.3	99.3±0.3
CD86+	69.1±6.1	73.6±5.3	70.0±6.6
CD163+	76.1±3.5	76.3±4.3	75.8±4.1
CD206+	78.0±5.3	80.1±4.5	74.1±9.1
P1&P2 gate (% positive cells):			
CD34+CD45^{dim}	0.25±0.08	0.25±0.07	0.27±0.09
CD34+CD45^{dim}KDR+	0.01±0.00	0.01±0.00	0.01±0.00
CD133+CD45^{dim}	0.09±0.06	0.06±0.04	0.06±0.05
CD133+CD45^{dim}KDR+	0.07±0.05	0.05±0.03	0.05±0.04
CD45+	94.8±2.3	94.8±2.4	93.8±2.7
P2 gate (% positive cells):			
CD3+CD19-	65.9±7.1	66.1±7.6	66.1±7.6
CD4+CD3+CD8-CD19-	29.5±4.8	31.0±4.0	29.8±4.7
CD8+CD3+CD4-CD19-	28.5±6.9	27.2±6.8	28.0±6.6
CD19+CD3-	6.1±2.6	6.0±2.5	6.6±3.1

EOCs indicate angiogenic early outgrowth cells; CHF, chronic heart failure; miR, microRNA. For gating strategy (P1 and P2 gates) please refer to supplemental figure 1. n=5-6 per group. Values are mean ± SEM. P = n.s. for all comparisons.

Supplemental Table 4. MicroRNA Array Profiling

Total RNA from sample and reference were labeled with Hy3™ and Hy5™ fluorescent label, respectively. The Hy3™-labelled samples and a Hy5™-labelled reference RNA sample were mixed pair-wise and hybridized to the miRCURY™ LNA array. After hybridization the microarray slides were scanned and the image analysis was carried out. 294 out of 757 miRNAs were found to be expressed in angiogenic EOCs. A, Normalized Hy3/Hy5 ratios (log2 transformed) from angiogenic EOCs from 4 healthy subjects. B, Median Hy3 signal from miRNAs and median Hy5 signal from reference of angiogenic EOCs from 4 healthy subjects.

A, Normalized Hy3/Hy5 ratios (log2 transformed)

	Healthy 1	Healthy 2	Healthy 3	Healthy 4	Mean values	SD
hsa-let-7f	NA	1.74	4.08	NA	2.9	1.9
hsa-miR-32	NA	2.16	4.46	0.82	2.48	1.84
hsa-miR-16	1.30	2.51	3.32	1.20	2.08	1.02
hsa-miR-20b	NA	1.99	3.68	0.47	2.05	1.60
hsa-miR-335	NA	0.50	3.32	NA	1.91	1.99
hsa-miR-301a	0.36	1.38	3.28	NA	1.68	1.48
hsa-miR-30e*	0.91	1.73	3.26	0.77	1.67	1.14
hsa-miR-142-3p	0.91	2.07	2.22	1.28	1.62	0.63
hsa-miR-27b	0.65	1.89	3.45	0.46	1.61	1.38
hsa-miR-30e	0.81	1.77	2.94	0.94	1.61	0.98
hsa-miR-374a	0.89	1.81	2.87	0.74	1.58	0.98
hsa-miR-140-5p	0.76	1.84	3.03	0.58	1.55	1.13
hsa-miR-19a	0.82	1.80	2.62	0.81	1.51	0.87
hsa-let-7a	0.62	2.01	2.98	0.42	1.51	1.21
hsa-miR-126	0.04	-0.11	5.17	0.77	1.47	2.50
hsa-miR-130a	0.28	0.29	4.19	0.65	1.35	1.90
hsa-miR-17	0.56	1.55	2.60	0.60	1.33	0.96
hsa-miR-106a	0.57	1.59	2.54	0.61	1.33	0.94
hsa-miR-98	NA	1.09	2.50	0.37	1.32	1.09
hsa-miR-29b	0.60	1.70	2.11	0.80	1.30	0.72
hsa-miR-18b	0.69	1.36	2.62	0.33	1.25	1.01
hsa-let-7g	0.24	1.60	2.73	0.41	1.25	1.16
hsa-miR-26b	0.52	1.53	2.01	0.81	1.22	0.68
hsa-miR-363*	0.99	1.07	1.82	0.85	1.18	0.43
hsa-miR-23b	0.74	1.23	2.30	0.44	1.18	0.81
hsa-miR-15a	0.82	1.49	1.95	0.44	1.17	0.68
hsa-miR-101	0.56	1.51	1.82	0.77	1.16	0.60
hsa-miR-29a*	0.69	1.42	1.93	0.58	1.15	0.64
hsa-miR-668	0.58	1.29	2.30	0.37	1.13	0.87
hsa-miR-15b	0.47	1.28	2.22	0.51	1.12	0.82

hsa-miR-154	0.55	1.23	2.38	0.32	1.12	0.93
hsa-miR-18a	0.55	1.36	2.41	0.11	1.11	1.01
hsa-miR-374b	0.44	1.26	2.44	0.27	1.10	0.99
hsa-miR-20a	0.39	1.33	2.20	0.45	1.09	0.85
hsa-miR-616*	0.92	NA	1.24	1.02	1.06	0.16
hsa-miR-768-5p	0.14	1.31	2.19	0.57	1.06	0.90
hsa-miR-33a	0.20	1.08	2.19	0.74	1.05	0.84
hsa-miR-105	0.73	1.53	1.80	0.13	1.05	0.76
hsa-miR-28-5p	0.35	1.00	2.58	0.26	1.05	1.07
hsa-miR-148a	NA	0.57	1.50	NA	1.03	0.66
hsa-miR-7	0.56	0.97	1.88	0.59	1.00	0.61
hsa-miR-195	0.36	0.98	2.34	0.30	1.00	0.95
hsa-miR-424	0.53	1.22	1.74	0.43	0.98	0.61
hsa-miR-342-3p	0.23	1.25	1.77	0.62	0.97	0.68
hsa-miR-483-3p	1.20	0.80	0.94	0.91	0.96	0.17
hsa-miR-223	0.73	1.14	1.82	0.07	0.94	0.73
hsa-miR-22*	0.47	1.10	1.90	0.17	0.91	0.77
hsa-miR-199a-5p	0.30	-0.30	2.79	0.85	0.91	1.34
hsa-miR-107	0.43	0.99	1.99	0.22	0.91	0.79
hsa-miR-150*	NA	0.60	1.21	NA	0.90	0.44
hsa-miR-194	0.40	0.92	1.84	0.45	0.90	0.67
hsa-miR-21	0.99	1.55	0.66	0.40	0.90	0.49
hsa-miR-30b	0.24	1.06	1.98	0.26	0.89	0.82
hsa-miR-338-3p	0.99	1.85	1.37	-0.68	0.88	1.10
hsa-let-7i	0.41	1.22	1.49	0.34	0.87	0.58
hsa-miR-27a	0.16	1.19	2.02	0.02	0.85	0.94
hsa-miR-34a	0.66	1.16	1.63	-0.08	0.85	0.73
miRPlus_17858	0.94	0.82	0.73	0.75	0.81	0.09
hsa-miR-31	0.31	1.09	1.48	0.27	0.79	0.59
hsa-miR-24	0.50	1.07	1.32	0.21	0.78	0.51
hsa-miR-221	0.24	0.50	1.92	0.42	0.77	0.77
hsa-miR-29c*	0.54	0.96	1.05	0.44	0.75	0.30
hsa-miR-19b	0.42	0.94	1.04	0.58	0.74	0.29
miRPlus_17841	0.62	0.24	1.70	0.38	0.74	0.66
hsa-miR-744	0.44	0.86	1.77	-0.15	0.73	0.81
hsa-miR-425	0.56	0.59	1.47	0.29	0.73	0.52
hsa-miR-103	0.50	0.86	1.29	0.21	0.71	0.47
hsa-miR-30c	0.29	0.76	1.42	0.30	0.69	0.54
hsa-miR-361-3p	0.28	0.81	1.18	0.47	0.68	0.39
hsa-miR-877*	0.83	0.66	0.52	0.69	0.68	0.13
hsa-miR-26b*	0.55	0.73	0.81	0.61	0.67	0.12
hsa-miR-342-5p	0.14	0.78	1.30	0.41	0.66	0.50
hsa-miR-652	0.29	0.52	1.10	NA	0.64	0.42
hsa-miR-181b	0.72	0.83	0.89	0.06	0.63	0.38
hsa-miR-30d	0.34	0.67	1.15	0.30	0.62	0.39
hsa-miR-361-5p	0.38	0.65	1.06	0.34	0.61	0.33
hsa-miR-339-5p	0.21	0.52	1.41	0.28	0.61	0.55
hsa-let-7d	0.05	0.63	1.75	-0.03	0.60	0.82
hsa-miR-186	0.27	0.49	1.18	0.46	0.60	0.40
hsa-miR-106b	0.38	0.72	0.97	0.29	0.59	0.31
hsa-miR-26a	0.36	0.74	0.66	0.53	0.57	0.17
hsa-miR-92a	0.18	0.62	1.03	0.41	0.56	0.36
hsa-miR-136	0.53	0.19	0.89	0.64	0.56	0.29
hsa-miR-338-5p	0.70	0.49	0.49	NA	0.56	0.12
hsa-miR-532-5p	0.55	0.62	0.88	0.19	0.56	0.28
hsa-miR-129*	0.49	0.43	1.45	-0.14	0.56	0.66
hsa-miR-365	0.31	1.07	0.57	0.22	0.54	0.38
hsa-miR-140-3p	0.23	0.58	1.08	0.27	0.54	0.39

hsa-miR-215	-0.01	0.55	1.46	0.14	0.53	0.66
hsa-miR-146b-5p	0.37	1.07	0.45	0.24	0.53	0.37
hsa-miR-331-3p	0.15	0.41	1.65	-0.09	0.53	0.77
hsa-let-7b	0.31	0.63	0.97	0.15	0.51	0.37
hsa-miR-484	0.34	0.46	1.10	0.13	0.51	0.42
hsa-miR-17*	0.31	0.55	0.92	0.22	0.50	0.31
hsa-miR-93	0.32	0.59	0.99	0.06	0.49	0.40
hsa-miR-150	0.06	0.57	0.93	0.38	0.49	0.37
hsa-miR-339-3p	NA	0.23	0.73	NA	0.48	0.35
hsa-miR-519d	0.64	0.52	0.54	0.17	0.47	0.21
hsa-miR-24-1*	NA	0.34	1.01	0.05	0.47	0.49
hsa-miR-377*	0.54	0.43	0.27	0.60	0.46	0.14
hsa-miR-340	0.36	0.37	0.80	0.19	0.43	0.26
hsa-miR-146a	0.21	0.95	0.43	0.10	0.42	0.38
hsa-miR-193a-3p	0.35	1.33	0.49	-0.52	0.42	0.76
hsa-miR-296-5p	0.53	0.66	0.16	0.30	0.41	0.22
hsa-miR-25	0.13	0.47	0.79	0.26	0.41	0.29
hsa-miR-92b	0.03	0.48	0.88	0.24	0.41	0.36
hsa-miR-222	-0.10	0.39	1.06	0.24	0.40	0.48
hsa-miR-500*	0.39	0.55	0.60	0.04	0.40	0.25
hsa-miR-147	0.12	0.35	0.96	0.14	0.39	0.39
hsa-miR-502-5p	0.27	0.31	0.55	0.44	0.39	0.13
hsa-miR-197	0.34	0.36	0.73	0.13	0.39	0.25
hsa-miR-502-3p	0.19	0.40	0.58	NA	0.39	0.19
hsa-miR-148b	0.19	0.29	0.91	0.15	0.39	0.36
hsa-miR-524-5p	0.28	0.57	0.62	0.05	0.38	0.27
hsa-miR-185	0.53	0.02	0.82	0.12	0.37	0.37
hsa-miR-26a-2*	0.17	0.33	0.43	0.52	0.36	0.15
hsa-miR-23a	0.63	0.37	0.46	-0.01	0.36	0.27
hsa-miR-362-5p	0.36	0.43	0.20	0.41	0.35	0.11
hsa-miR-191	0.31	0.44	0.61	0.03	0.35	0.24
hsa-miR-423-3p	0.20	0.29	0.96	-0.10	0.34	0.45
hsa-miR-138-1*	0.09	0.41	0.81	0.03	0.33	0.35
hsa-miR-220b	0.49	0.31	0.34	0.19	0.33	0.13
hsa-miR-620	0.24	0.32	0.55	0.09	0.30	0.19
hsa-miR-125b	-0.18	0.52	0.38	0.41	0.28	0.31
hsa-miR-142-5p	0.27	0.36	-0.07	0.56	0.28	0.26
hsa-miR-378	0.71	-0.36	0.21	0.52	0.27	0.47
hsa-miR-500	0.52	0.24	0.12	0.12	0.25	0.19
hsa-miR-612	0.34	0.45	0.04	0.15	0.24	0.19
hsa-miR-337-3p	0.24	0.12	0.37	0.22	0.24	0.10
hsa-miR-629	0.15	0.19	0.37	NA	0.23	0.12
hsa-miR-29a	0.08	0.49	-0.02	0.39	0.23	0.24
hsa-miR-519e	0.19	0.25	0.25	0.18	0.22	0.03
hsa-miR-125a-5p	0.04	0.98	0.12	-0.31	0.21	0.55
hsa-miR-34b	0.05	0.03	0.47	0.28	0.21	0.21
miRPlus_17892	0.35	0.21	0.02	0.20	0.20	0.14
hsa-let-7c	0.11	0.15	0.45	0.05	0.19	0.18
hsa-miR-623	0.25	0.19	-0.05	0.37	0.19	0.17
hsa-miR-505	-0.07	0.16	0.47	NA	0.19	0.27
hsa-miR-574-3p	0.20	0.17	0.30	0.06	0.18	0.10
hsa-miR-615-3p	0.36	0.05	-0.04	0.29	0.16	0.19
hsa-miR-20b*	0.14	NA	0.27	0.09	0.16	0.09
hsa-miR-486-5p	-0.04	-0.18	0.65	0.21	0.16	0.36
hsa-miR-886-5p	-0.13	-0.51	1.04	0.22	0.16	0.66
hsa-miR-99b	0.11	1.20	0.17	-0.88	0.15	0.85
hsa-miR-130b	0.05	-0.04	0.51	0.04	0.14	0.25
hsa-miR-886-3p	-0.41	-0.45	0.78	0.62	0.14	0.66

hsa-miR-151-3p	-0.05	-0.49	0.84	0.25	0.13	0.56
hsa-miR-374b*	0.24	0.08	0.01	0.18	0.13	0.10
hsa-miR-202	-0.10	0.17	0.30	NA	0.12	0.21
hsa-let-7e	-0.11	0.22	0.42	-0.05	0.12	0.25
hsa-miR-106b*	0.04	0.00	0.35	0.05	0.11	0.16
hsa-miR-768-3p	0.01	0.23	-0.45	0.52	0.08	0.41
hsa-miR-425*	0.03	-0.05	0.24	NA	0.08	0.15
hsa-miR-181a	0.50	0.33	-0.37	-0.17	0.07	0.41
hsa-miR-297	0.36	-0.07	-0.27	0.24	0.06	0.29
hsa-miR-125a-3p	0.19	0.29	-0.12	-0.13	0.05	0.21
hsa-miR-22	0.38	0.22	-0.49	0.07	0.04	0.38
hsa-miR-1	-0.01	-0.17	0.40	-0.09	0.03	0.25
hsa-miR-518a-3p	0.15	-0.04	-0.07	0.03	0.02	0.10
hsa-miR-629*	0.13	0.06	-0.05	-0.07	0.02	0.09
hsa-miR-129-5p	-0.03	0.70	-0.33	-0.33	0.00	0.48
hsa-miR-720	0.56	0.07	-1.04	0.42	0.00	0.72
hsa-let-7d*	0.15	-0.16	-0.09	0.10	0.00	0.15
miRPlus_42780	0.02	-0.54	1.00	-0.49	0.00	0.72
hsa-miR-625*	0.03	0.30	-0.22	-0.15	-0.01	0.23
hsa-let-7b*	-0.14	-0.05	0.26	-0.12	-0.01	0.19
hsa-miR-600	-0.28	-0.03	0.51	-0.26	-0.01	0.37
hsa-miR-943	0.07	0.01	-0.05	-0.18	-0.04	0.10
miRPlus_42856	0.19	-0.25	-0.33	0.20	-0.05	0.28
hsa-miR-585	0.07	0.50	-0.38	-0.41	-0.05	0.43
hsa-miR-487b	-0.04	-0.02	0.04	-0.22	-0.06	0.11
hsa-miR-302c*	0.07	-0.06	-0.18	-0.10	-0.07	0.10
hsa-miR-320a	0.20	-0.36	-0.27	0.15	-0.07	0.29
hsa-miR-622	-0.01	NA	-0.23	0.01	-0.08	0.13
hsa-miR-147b	-0.09	0.98	-1.15	NA	-0.09	1.07
hsa-miR-509-3-5p	0.21	-0.18	-0.54	0.04	-0.12	0.32
hsa-miR-940	-0.07	0.34	-0.65	NA	-0.13	0.49
hsa-miR-549	-0.03	-0.38	-0.22	0.04	-0.15	0.19
hsa-miR-553	-0.01	-0.32	NA	-0.11	-0.15	0.16
hsa-miR-584	0.01	-0.01	-0.19	-0.41	-0.15	0.19
hsa-miR-146b-3p	0.05	-0.21	-0.54	0.04	-0.16	0.28
hsa-miR-550	-0.19	0.06	-0.66	0.14	-0.16	0.36
hsa-miR-887	NA	-0.19	-0.06	-0.28	-0.18	0.11
hsa-miR-625	-0.24	-0.52	0.30	-0.28	-0.18	0.35
hsa-miR-637	-0.13	-0.23	-0.18	-0.29	-0.21	0.07
hsa-miR-300	0.03	-0.30	-0.25	-0.33	-0.21	0.16
hsa-miR-885-5p	0.01	-0.36	-0.51	-0.01	-0.22	0.26
hsa-miR-23a*	-0.23	-0.26	-0.17	NA	-0.22	0.05
hsa-miR-576-3p	0.12	-0.50	-0.75	0.17	-0.24	0.46
hsa-miR-369-3p	0.26	-0.33	-1.07	0.11	-0.26	0.60
hsa-miR-181a-2*	NA	-0.43	-0.02	-0.33	-0.26	0.22
hsa-miR-485-3p	-0.25	-0.35	-0.34	-0.16	-0.28	0.09
hsa-miR-518b	-0.07	0.08	-0.70	-0.43	-0.28	0.35
hsa-miR-99b*	-0.30	-0.56	-0.36	0.06	-0.29	0.26
hsa-miR-766	-0.22	0.16	-0.63	-0.50	-0.30	0.35
hsa-miR-185*	-0.28	-0.59	-0.18	-0.15	-0.30	0.20
hsa-miR-630	-0.12	-0.40	-0.64	-0.08	-0.31	0.26
hsa-miR-21*	-0.28	-0.05	-0.53	-0.39	-0.31	0.20
hsa-miR-125b-1*	-0.17	-0.43	-0.52	-0.20	-0.33	0.17
hsa-miR-505*	-0.09	-0.55	-0.50	-0.18	-0.33	0.23
hsa-miR-382	-0.18	-0.54	-0.43	-0.17	-0.33	0.18
hsa-miR-617	-0.02	-0.51	-0.60	-0.22	-0.34	0.26
miRPlus_42521	-0.19	0.03	-0.87	-0.35	-0.34	0.38
hsa-miR-934	-0.13	-0.47	-0.71	-0.14	-0.36	0.28

hsa-miR-488	-0.11	-0.43	-0.70	-0.22	-0.36	0.26
hsa-miR-302d*	-0.11	-0.37	-0.71	-0.29	-0.37	0.25
hsa-miR-423-5p	-0.25	-0.53	-0.64	-0.12	-0.38	0.24
hsa-miR-891a	-0.33	-0.53	-0.41	-0.27	-0.39	0.11
hsa-miR-124	-0.57	-0.61	-0.49	0.13	-0.39	0.35
hsa-miR-634	-0.23	-0.32	-0.64	-0.36	-0.39	0.17
hsa-miR-596	-0.20	0.15	-0.88	-0.62	-0.39	0.46
hsa-miR-877	-0.28	-0.67	-0.37	-0.24	-0.39	0.20
hsa-miR-642	-0.05	-0.10	-0.97	-0.54	-0.42	0.43
hsa-miR-206	-0.20	-0.62	-0.66	-0.24	-0.43	0.24
hsa-miR-185	-0.05	-0.78	-0.73	-0.16	-0.43	0.38
hsa-miR-516b	-0.25	-0.59	-0.69	-0.20	-0.43	0.24
hsa-miR-184	-0.34	-0.49	-0.87	-0.06	-0.44	0.34
hsa-miR-187*	-0.23	-0.48	-0.89	-0.22	-0.46	0.32
hsa-miR-155	-0.10	-0.69	-0.90	-0.18	-0.47	0.39
hsa-miR-638	-0.43	0.27	-1.06	-0.69	-0.48	0.56
hsa-miR-526b	-0.10	-0.65	-0.99	-0.18	-0.48	0.42
hsa-miR-671-5p	-0.49	-0.33	-0.68	-0.43	-0.48	0.15
hsa-miR-214	-0.31	-0.56	-0.90	-0.17	-0.49	0.32
hsa-miR-299-3p	-0.18	-0.79	-0.83	-0.14	-0.49	0.38
hsa-miR-525-5p	-0.26	-0.21	-1.01	-0.47	-0.49	0.37
hsa-miR-409-5p	0.00	-0.57	-1.26	-0.15	-0.49	0.56
hsa-miR-513a-5p	0.02	-0.55	-1.35	-0.11	-0.50	0.62
hsa-miR-198	-0.42	-0.62	-0.83	-0.17	-0.51	0.28
hsa-miR-498	-0.33	-0.40	-0.94	-0.39	-0.52	0.29
hsa-miR-519e*	-0.25	-0.65	-0.81	-0.36	-0.52	0.26
hsa-miR-509-5p	-0.19	-0.70	-0.93	-0.27	-0.53	0.35
hsa-miR-326	-0.41	-1.00	-0.29	-0.44	-0.53	0.32
hsa-miR-510	-0.29	-0.64	-0.92	-0.29	-0.54	0.30
hsa-miR-574-5p	-0.09	-0.78	-1.19	-0.09	-0.54	0.54
hsa-miR-483-5p	-0.33	-0.79	-0.86	-0.22	-0.55	0.32
hsa-miR-659	-0.32	-0.70	-0.93	-0.28	-0.56	0.31
hsa-miR-422a	-0.19	-0.63	-1.18	-0.25	-0.56	0.46
hsa-miR-503	-0.32	-0.58	-1.16	-0.22	-0.57	0.42
hsa-miR-200b*	-0.37	-0.31	-1.15	-0.46	-0.57	0.39
miRPlus_17955	-0.39	-0.45	-1.03	-0.44	-0.57	0.30
hsa-miR-490-3p	-0.17	-0.78	-1.13	-0.23	-0.58	0.46
miRPlus_42526	-0.27	-0.46	-1.67	0.08	-0.58	0.76
hsa-miR-25*	-0.49	-0.54	-0.78	-0.52	-0.58	0.14
hsa-miR-183*	-0.53	-0.39	-0.84	-0.56	-0.58	0.19
hsa-miR-193b*	-0.48	-0.80	-0.87	-0.23	-0.60	0.29
hsa-miR-551a	-0.40	-0.84	-0.98	-0.21	-0.61	0.37
hsa-miR-620	0.35	-0.94	-1.86	-0.04	-0.63	0.99
hsa-miR-937	-0.56	-0.76	-0.78	-0.45	-0.64	0.16
hsa-miR-516a-5p	-0.45	-1.01	-0.73	-0.36	-0.64	0.29
hsa-miR-492	-0.87	-0.16	-0.99	-0.54	-0.64	0.37
hsa-miR-519c-5p/hsa-miR-519b-5p/hsa-miR-523*/hsa-miR-518e*/hsa-miR-522*/hsa-miR-519a*	-0.46	-0.48	-1.16	-0.48	-0.64	0.34
hsa-miR-628-3p	-0.42	-0.45	-1.32	-0.54	-0.68	0.43
hsa-miR-551b*	-0.19	-0.88	-1.23	-0.46	-0.69	0.46
hsa-miR-933	0.03	-0.77	-1.83	-0.19	-0.69	0.83
miRPlus_42793	-0.48	-0.93	-1.04	-0.33	-0.69	0.34
hsa-miR-526b*	-0.48	-0.86	-1.00	-0.53	-0.72	0.25

hsa-miR-765	-0.04	-1.01	-1.68	-0.14	-0.72	0.78
hsa-miR-663	-0.68	-0.32	-1.48	-0.42	-0.72	0.52
hsa-miR-520d-5p	-0.38	-0.86	-1.11	-0.55	-0.73	0.33
hsa-miR-939	-0.33	-0.85	-1.41	-0.33	-0.73	0.52
miRPlus_42745	-0.36	0.12	-1.81	-0.89	-0.73	0.83
hsa-miR-518a-5p/hsa-miR-527	-0.35	-0.89	-1.16	-0.54	-0.74	0.36
hsa-miR-193a-5p	-0.24	-0.16	-1.85	-0.70	-0.74	0.78
hsa-miR-10a*	-0.44	-0.87	-1.09	-0.58	-0.75	0.29
hsa-miR-923	0.58	-1.09	-3.27	0.76	-0.76	1.87
miRPlus_42487	-0.16	-0.27	-2.33	-0.29	-0.76	1.04
hsa-miR-583	-0.45	-1.14	-1.16	-0.34	-0.77	0.44
miRPlus_27560	-0.98	-0.84	-0.95	-0.34	-0.78	0.30
hsa-miR-542-5p	-0.50	-1.22	-1.06	-0.32	-0.78	0.43
hsa-miR-149*	-0.71	-0.77	-1.17	-0.51	-0.79	0.28
hsa-miR-518c*	-0.32	-1.07	-1.54	-0.26	-0.80	0.62
hsa-miR-874	-0.38	0.09	-1.81	-1.12	-0.80	0.83
miRPlus_17869	-0.38	-1.12	-1.50	-0.24	-0.81	0.60
hsa-miR-32*	-0.11	-1.07	-1.99	-0.13	-0.83	0.90
miRPlus_28431	-0.50	-0.35	-1.87	-0.64	-0.84	0.70
hsa-miR-298	0.17	-1.12	-2.16	-0.26	-0.84	1.03
hsa-miR-675	-0.84	-0.61	-1.07	-0.87	-0.85	0.19
hsa-miR-7-2*	-0.57	-1.06	-1.32	-0.51	-0.87	0.39
hsa-miR-658	-0.43	-1.09	-1.59	-0.42	-0.88	0.57
hsa-miR-30b*	-0.60	-0.85	-1.82	-0.39	-0.91	0.63
hsa-miR-557	-0.58	-0.35	-1.83	-0.92	-0.92	0.65
hsa-miR-30c-1*	-0.64	-1.28	-1.24	-0.69	-0.96	0.35
hsa-miR-665	-0.51	-1.06	-2.05	-0.44	-1.01	0.74
hsa-miR-30c-2*	-0.53	-1.52	-1.76	-0.36	-1.04	0.70
hsa-miR-602	-0.54	-1.03	-2.13	-0.59	-1.07	0.74
hsa-miR-551b	-0.56	-1.45	-1.85	-0.48	-1.09	0.67
hsa-miR-640	-0.81	-1.27	-1.54	-0.73	-1.09	0.39
hsa-miR-631	-0.84	-1.39	-1.68	-0.70	-1.15	0.46
hsa-miR-552	-0.50	-1.56	-2.19	-0.38	-1.16	0.87
hsa-miR-371-5p	-0.55	-0.31	-2.71	-1.09	-1.17	1.08
hsa-miR-921	-0.53	-1.48	-2.26	-0.42	-1.17	0.87
hsa-miR-381	-0.43	-1.57	-2.34	-0.45	-1.20	0.93
hsa-miR-494	-0.31	-1.68	-3.04	-0.43	-1.37	1.28
miRPlus_17952	-1.28	-3.28	-5.31	-1.15	-2.75	1.96

miR indicates microRNA; SD, standard deviation;
A particular microRNA that failed on an array is indicated by the acronym "NA".

B, Median Hy3 and Hy5 signal

	Healthy 1		Healthy 2		Healthy 3		Healthy 4	
	median Hy3	median Hy5	median Hy3	median Hy5	median Hy3	median Hy5	median Hy3	median Hy5
hsa-let-7f	46.9	35.0	136.6	40.2	518.3	29.4	44.0	39.3
hsa-miR-32	48.8	33.3	141.7	31.5	462.2	21.3	65.4	37.1
hsa-miR-16	1039.0	420.6	2925.1	510.3	5775.3	581.0	744.0	320.5
hsa-miR-20b	44.9	39.3	156.6	39.3	377.2	29.5	50.3	36.9
hsa-miR-335	39.2	43.9	68.1	47.8	319.5	32.4	42.7	39.8
hsa-miR-301a	50.5	39.9	96.6	37.9	294.8	30.5	45.4	35.5
hsa-miR-30e*	87.3	46.4	177.8	53.3	390.9	42.4	88.9	52.3
hsa-miR-142-3p	5425.4	2932.4	16290.7	3935.1	20887.8	4422.5	6107.7	2546.1
hsa-miR-27b	305.5	192.9	880.0	238.3	2266.3	207.2	245.9	177.6
hsa-miR-30e	450.2	254.5	1088.3	319.6	2250.4	285.8	464.9	239.7
hsa-miR-374a	382.9	206.3	894.6	255.7	1883.1	261.7	294.8	171.9
hsa-miR-140-5p	90.3	53.3	216.6	59.8	401.8	49.0	148.8	103.9
hsa-miR-19a	546.9	313.5	1396.0	404.2	2140.0	342.2	566.9	329.6
hsa-let-7a	352.6	228.0	989.1	257.1	2209.3	280.1	239.7	179.3
hsa-miR-126	63.7	62.4	68.2	74.3	2051.4	57.7	86.4	50.2
hsa-miR-130a	62.8	51.7	73.1	59.1	801.3	43.6	75.3	46.8
hsa-miR-17	266.7	177.5	657.1	224.3	1299.6	215.6	229.8	151.8
hsa-miR-106a	266.9	181.3	647.3	214.2	1244.8	214.3	219.2	142.7
hsa-miR-98	47.4	41.3	97.5	46.3	216.3	38.2	51.0	40.0
hsa-miR-29b	1549.9	1047.7	4216.2	1337.6	5190.3	1174.9	1675.1	989.2
hsa-miR-18b	97.2	60.2	176.4	68.3	324.2	52.7	70.4	56.2
hsa-let-7g	117.3	99.2	312.7	103.2	584.3	86.6	117.5	89.6
hsa-miR-26b	1803.5	1260.1	4652.7	1605.0	8389.6	2047.6	1709.3	985.4
hsa-miR-363*	69.1	33.5	94.8	44.8	128.4	36.6	72.5	40.9
hsa-miR-23b	1049.8	645.6	2091.5	888.2	4705.8	960.3	833.6	611.5
hsa-miR-15a	777.5	450.6	1622.4	568.9	2275.3	590.0	500.0	372.3
hsa-miR-101	772.1	523.2	1864.0	657.8	2378.3	682.2	744.0	436.0
hsa-miR-29a*	74.5	46.3	135.5	50.4	174.8	44.7	70.8	47.5
hsa-miR-668	148.8	100.8	291.9	119.7	479.5	97.6	119.7	95.3
hsa-miR-15b	416.9	298.3	915.5	377.4	1802.6	384.1	355.8	251.0
hsa-miR-154	241.7	167.3	491.8	204.3	952.2	182.7	188.9	149.7
hsa-miR-18a	93.9	64.0	180.5	71.1	342.5	64.0	60.5	55.9
hsa-miR-374b	105.3	78.6	212.6	90.5	380.4	69.2	90.3	77.1
hsa-miR-20a	499.8	376.9	1125.6	450.2	2243.4	482.2	440.0	318.1

hsa-miR-616*	51.1	27.2	51.3	24.4	51.2	22.9	52.4	25.6
hsa-miR-768-5p	402.4	368.8	1065.6	434.8	2118.0	469.4	436.0	291.3
hsa-miR-33a	68.2	58.1	138.8	66.3	262.4	56.6	92.7	53.5
hsa-miR-105	226.0	136.7	445.1	155.5	442.6	126.0	142.8	131.0
hsa-miR-28-5p	82.7	65.9	147.2	73.4	360.1	60.6	68.2	57.2
hsa-miR-148a	46.2	47.8	71.3	48.0	115.8	40.7	48.0	43.7
hsa-miR-7	53.3	37.1	76.2	39.0	116.1	32.1	55.7	35.8
hsa-miR-195	54.9	43.5	98.0	49.9	188.1	37.6	55.0	46.1
hsa-miR-424	158.7	109.9	299.3	127.2	417.6	125.2	128.0	94.5
hsa-miR-342-3p	632.4	533.4	1597.1	679.5	2434.7	735.9	672.8	437.0
hsa-miR-483-3p	91.0	39.6	67.0	39.1	62.0	31.6	61.3	32.2
hsa-miR-223	2003.7	1209.9	3303.5	1502.8	6544.9	1854.4	999.2	938.8
hsa-miR-22*	89.0	63.1	149.9	68.9	221.0	59.2	69.7	62.0
hsa-miR-199a-5p	66.7	54.0	53.0	65.0	358.2	52.4	75.9	44.4
hsa-miR-107	216.9	162.4	394.6	201.5	665.4	167.2	181.8	159.6
hsa-miR-150*	46.0	42.5	72.9	48.6	104.4	43.6	44.8	43.9
hsa-miR-194	56.5	42.7	104.0	54.9	152.1	43.1	65.6	48.2
hsa-miR-21	6332.2	3176.6	12406.8	4284.8	8700.8	5523.0	3606.1	2806.7
hsa-miR-30b	631.0	532.6	1380.5	657.7	2749.2	697.3	537.9	448.0
hsa-miR-338-3p	246.9	123.2	480.5	137.4	306.2	116.9	74.5	116.1
hsa-let-7i	546.1	417.5	1227.1	541.7	1570.8	556.5	450.7	356.5
hsa-miR-27a	697.4	628.6	1734.6	756.0	3130.4	781.3	552.1	543.1
hsa-miR-34a	283.8	169.8	436.7	196.4	560.6	178.9	135.8	145.3
miRPlus_17858	81.2	42.9	74.4	41.9	68.1	41.1	58.4	33.8
hsa-miR-31	71.8	58.2	133.0	62.8	160.9	58.4	60.6	50.3
hsa-miR-24	1516.8	1043.5	2804.1	1334.7	4073.4	1647.6	1039.5	887.0
hsa-miR-221	165.9	140.5	239.8	169.1	549.9	141.5	187.1	137.2
hsa-miR-29c*	65.3	45.0	95.6	48.8	97.4	46.6	59.5	42.6
hsa-miR-19b	1136.7	825.3	2029.0	1081.5	2131.0	1061.2	1198.5	802.9
miRPlus_17841	65.3	42.9	62.0	52.9	137.6	41.8	58.3	44.6
hsa-miR-744	97.3	70.6	145.5	80.3	255.1	73.8	59.5	66.1
hsa-miR-425	169.9	113.5	196.9	128.3	325.0	116.3	125.7	102.8
hsa-miR-103	576.6	405.4	848.5	465.8	1225.7	490.4	383.7	332.8
hsa-miR-30c	647.8	530.1	1061.2	626.0	1720.1	641.7	533.3	445.8
hsa-miR-361-3p	127.1	105.6	203.8	115.8	222.2	95.8	136.3	97.5
hsa-miR-877*	60.7	33.8	62.4	38.5	51.7	36.0	53.3	33.0
hsa-miR-26b*	56.2	39.4	71.0	42.9	73.6	42.0	56.9	36.7
hsa-miR-342-5p	101.4	92.2	172.8	100.8	232.1	95.6	105.7	76.2

hsa-miR-652	57.6	47.0	77.4	53.7	110.5	51.0	40.0	45.7
hsa-miR-181b	165.2	99.3	205.9	114.0	176.6	94.7	108.4	104.7
hsa-miR-30d	405.3	319.3	591.2	378.1	798.3	359.5	328.2	262.9
hsa-miR-361-5p	313.2	241.4	425.6	272.0	554.5	264.1	243.5	191.0
hsa-miR-339-5p	198.5	173.5	287.0	199.9	518.2	197.5	165.1	135.5
hsa-let-7d	100.0	96.8	167.8	109.0	324.5	96.4	90.0	91.7
hsa-miR-186	212.1	178.8	300.3	215.4	428.0	190.5	204.8	151.0
hsa-miR-106b	823.4	633.7	1269.6	755.8	1729.5	881.0	578.4	464.4
hsa-miR-26a	2510.5	1972.9	4616.7	2693.1	5299.5	3385.0	2363.6	1623.2
hsa-miR-92a	111.2	97.5	158.9	102.8	188.6	92.1	122.1	91.9
hsa-miR-136	78.3	54.2	69.1	60.5	96.9	52.2	80.6	51.1
hsa-miR-338-5p	61.2	38.4	63.4	46.2	58.0	41.8	48.6	38.0
hsa-miR-532-5p	71.2	51.1	89.1	58.5	91.3	50.8	54.0	47.6
hsa-miR-129*	77.5	55.9	80.8	59.0	134.4	49.8	49.3	54.4
hsa-miR-365	62.5	49.2	124.2	59.0	79.0	53.2	104.0	78.3
hsa-miR-140-3p	529.0	452.3	786.2	521.9	1134.7	537.7	427.8	350.0
hsa-miR-215	73.9	75.4	126.3	87.5	179.7	68.8	94.0	84.7
hsa-miR-146b-5p	1268.3	994.0	2571.8	1195.4	1870.1	1372.7	871.4	755.9
hsa-miR-331-3p	81.8	71.3	110.4	83.1	231.6	74.1	62.4	66.5
hsa-let-7b	289.9	236.5	436.5	278.9	560.6	283.1	202.2	182.0
hsa-miR-484	78.5	65.5	97.4	71.1	135.6	65.4	62.6	58.1
hsa-miR-17*	72.7	59.5	102.1	69.3	115.5	61.8	68.3	58.9
hsa-miR-93	312.1	250.9	441.6	293.8	594.0	293.1	225.2	216.5
hsa-miR-150	769.1	704.6	1358.4	932.3	1791.3	943.6	760.5	590.8
hsa-miR-339-3p	43.1	47.1	65.4	55.0	78.6	47.7	38.0	43.8
hsa-miR-519d	708.2	454.9	775.3	532.8	724.4	496.3	476.0	427.4
hsa-miR-24-1*	117.4	108.5	143.9	113.5	209.4	106.3	105.7	100.7
hsa-miR-377*	56.0	38.5	64.5	46.9	56.3	45.8	70.9	46.8
hsa-miR-340	375.9	295.8	456.1	355.8	541.1	311.5	305.5	266.7
hsa-miR-146a	706.8	609.9	1384.1	720.2	1040.0	773.1	516.5	485.9
hsa-miR-193a-3p	172.8	136.7	399.8	156.5	203.8	144.1	80.7	113.9
hsa-miR-296-5p	59.7	42.9	77.1	50.1	52.5	47.1	164.0	132.7
hsa-miR-25	269.7	242.7	392.8	285.8	444.9	259.5	253.9	212.4
hsa-miR-92b	63.1	62.9	97.1	68.4	108.0	58.3	70.2	61.1
hsa-miR-222	354.2	379.9	585.5	443.0	934.0	451.2	375.5	313.7
hsa-miR-500*	101.2	78.2	128.6	88.9	124.9	83.8	74.5	72.9
hsa-miR-147	57.8	54.9	78.4	60.6	102.7	51.7	58.3	52.5
hsa-miR-502-5p	51.8	42.7	56.7	45.9	55.5	38.6	54.4	39.8

hsa-miR-197	129.0	102.8	141.3	108.9	165.0	101.0	99.1	91.6
hsa-miR-502-3p	65.4	57.4	83.7	64.7	84.4	55.9	47.3	51.7
hsa-miR-148b	264.5	233.4	336.3	278.7	484.3	258.7	207.7	185.4
hsa-miR-524-5p	130.8	107.6	186.0	125.9	165.8	107.8	110.3	104.8
hsa-miR-185	484.1	337.9	390.2	388.6	695.3	397.4	292.4	269.3
hsa-miR-26a-2*	90.7	81.3	112.6	89.0	117.0	87.6	94.5	68.3
hsa-miR-23a	2848.4	1832.4	3082.9	2361.5	4209.0	3087.5	1440.2	1455.3
hsa-miR-362-5p	71.8	55.7	87.1	64.5	71.7	62.2	116.6	65.0
hsa-miR-191	1016.4	822.8	1435.2	1063.5	1790.0	1153.8	667.9	657.4
hsa-miR-423-3p	309.0	268.2	402.1	328.1	594.6	307.0	215.3	228.9
hsa-miR-138-1*	193.4	182.2	296.6	226.0	318.5	182.3	203.9	199.9
hsa-miR-220b	84.7	60.6	93.4	78.6	82.6	67.5	74.8	66.0
hsa-miR-620	311.1	262.8	343.1	280.7	375.4	256.8	302.3	279.6
hsa-miR-125b	59.9	67.9	117.4	83.1	96.0	74.3	108.7	80.9
hsa-miR-142-5p	7052.0	5871.2	11634.5	9239.2	11707.6	12362.2	6831.6	4618.1
hsa-miR-378	569.2	352.4	331.8	427.9	479.7	408.8	394.7	275.0
hsa-miR-500	73.8	51.5	74.0	59.5	64.2	59.3	51.7	47.9
hsa-miR-612	60.7	45.5	74.0	53.4	51.8	51.6	53.3	49.3
hsa-miR-337-3p	56.2	47.5	56.7	54.9	62.4	48.6	57.8	49.7
hsa-miR-629	55.1	49.5	63.9	57.5	66.9	51.8	48.6	53.2
hsa-miR-29a	4545.9	4342.5	8192.9	5940.8	7857.6	7686.1	4484.4	3451.1
hsa-miR-519e	54.0	47.8	65.4	56.4	65.4	54.8	51.6	45.3
hsa-miR-125a-5p	574.9	560.4	1296.9	654.7	760.7	688.8	379.3	469.3
hsa-miR-34b	623.5	615.9	703.7	684.7	932.1	681.9	665.4	555.7
miRPlus_17892	67.0	52.4	66.4	57.8	55.6	53.9	58.9	50.4
hsa-let-7c	555.0	521.9	746.9	668.2	928.7	680.3	506.5	488.2
hsa-miR-623	61.5	51.5	63.5	56.1	53.9	55.2	63.8	49.9
hsa-miR-505	57.4	60.2	75.9	69.0	86.5	62.6	46.7	54.7
hsa-miR-574-3p	154.1	135.5	173.4	154.9	167.3	136.3	133.8	127.8
hsa-miR-615-3p	73.3	59.2	67.7	65.1	62.9	63.6	69.8	56.3
hsa-miR-20b*	90.7	83.6	128.6	88.9	97.3	80.8	89.9	83.1
hsa-miR-486-5p	52.9	54.0	53.8	61.3	84.4	54.8	53.5	46.2
hsa-miR-886-5p	79.8	89.7	76.2	109.4	187.3	91.7	107.0	85.4
hsa-miR-99b	154.4	149.8	370.5	162.6	170.5	152.8	69.6	129.3
hsa-miR-130b	112.0	108.4	123.0	122.4	156.4	110.0	104.1	102.1
hsa-miR-886-3p	159.8	210.2	184.4	251.9	414.3	244.6	248.7	165.3
hsa-miR-151-3p	63.7	65.8	54.2	76.5	121.7	68.2	67.4	58.0
hsa-miR-374b*	118.9	100.7	115.5	110.0	111.1	110.6	99.6	90.2

hsa-miR-202	53.4	59.1	78.9	69.1	73.6	57.8	45.6	64.8
hsa-let-7e	830.1	895.9	1228.2	1065.2	1516.2	1133.5	810.7	858.1
hsa-miR-106b*	69.2	67.5	83.5	79.6	93.2	73.0	72.1	69.6
hsa-miR-768-3p	3635.8	3600.4	5290.9	4555.6	4396.6	6145.3	3898.9	2708.7
hsa-miR-425*	57.0	56.5	56.9	58.8	64.7	54.6	48.4	51.8
hsa-miR-181a	1057.5	737.9	1188.6	962.8	687.1	889.6	683.3	777.6
hsa-miR-297	117.7	94.6	100.8	105.3	74.3	87.6	117.5	100.6
hsa-miR-125a-3p	60.9	53.8	81.8	66.4	56.3	61.4	55.3	58.8
hsa-miR-22	3179.8	2510.1	3712.9	3183.4	3048.8	4281.3	2116.7	1995.8
hsa-miR-1	147.5	147.8	150.4	167.7	172.7	131.0	164.1	177.4
hsa-miR-518a-3p	54.4	48.9	54.9	58.2	50.0	53.5	49.6	53.9
hsa-miR-629*	59.7	54.7	62.7	60.7	53.5	55.9	53.8	56.2
hsa-miR-129-5p	225.2	228.7	394.7	247.8	170.6	210.9	188.8	235.3
hsa-miR-720	9025.5	6184.3	8810.5	8035.6	6341.5	13072.4	6212.1	4667.8
hsa-let-7d*	91.5	81.3	88.0	98.1	80.2	85.6	85.5	76.7
miRPlus_42780	83.2	83.1	66.7	96.6	167.2	83.5	52.8	73.8
hsa-miR-625*	259.9	253.4	352.2	283.6	212.9	253.5	217.6	240.3
hsa-let-7b*	48.2	54.9	58.9	61.0	66.9	55.7	49.5	53.0
hsa-miR-600	55.8	69.8	72.9	74.6	84.1	59.3	57.4	68.6
hsa-miR-943	118.3	109.5	132.9	131.7	101.9	105.0	101.9	111.3
miRPlus_42856	983.1	845.3	871.1	1039.9	877.0	1096.5	811.7	682.8
hsa-miR-585	72.9	70.1	91.0	64.3	48.3	62.2	63.0	84.4
hsa-miR-487b	239.3	245.2	287.5	287.2	255.6	249.0	213.4	246.8
hsa-miR-302c*	61.4	58.8	65.7	69.0	53.6	60.8	60.1	62.7
hsa-miR-320a	839.2	750.2	714.5	913.5	818.9	993.5	623.0	568.8
hsa-miR-622	58.9	59.1	47.4	68.7	48.4	57.1	60.4	60.1
hsa-miR-147b	62.0	66.7	140.6	70.4	38.0	84.6	38.6	58.7
hsa-miR-509-3-5p	260.4	225.0	237.7	258.9	158.6	227.8	215.9	201.1
hsa-miR-940	60.6	62.5	89.2	67.5	43.2	69.9	41.3	60.9
hsa-miR-549	505.2	514.9	459.3	586.9	478.6	552.5	451.4	444.0
hsa-miR-553	53.7	54.9	52.4	66.8	44.6	58.4	51.9	55.5
hsa-miR-584	171.0	167.1	192.6	192.2	140.9	161.1	123.1	163.4
hsa-miR-146b-3p	189.5	182.9	175.2	201.9	122.1	175.8	170.2	165.0
hsa-miR-550	3098.9	3512.3	4805.2	4687.0	3900.4	6078.0	2827.0	2596.0
hsa-miR-887	188.2	184.3	180.5	205.7	171.5	179.3	149.3	180.8
hsa-miR-625	122.9	145.0	118.7	170.0	181.7	146.0	122.5	147.4
hsa-miR-637	109.1	119.3	106.2	123.6	95.3	108.7	101.4	124.1
hsa-miR-300	530.5	517.7	488.8	598.1	478.0	569.4	385.2	481.9

hsa-miR-885-5p	121.1	121.1	112.3	138.1	87.8	128.4	114.2	113.0
hsa-miR-23a*	45.9	54.6	54.5	64.6	50.9	57.5	45.5	53.8
hsa-miR-576-3p	599.4	544.3	423.8	573.1	311.6	524.1	591.2	518.3
hsa-miR-369-3p	97.2	81.8	74.1	93.8	43.7	90.1	93.7	86.7
hsa-miR-181a-2*	61.7	83.8	52.7	71.1	60.4	61.2	54.6	74.2
hsa-miR-485-3p	112.2	132.5	113.8	145.1	113.4	141.9	106.8	118.0
hsa-miR-518b	76.1	82.5	94.3	89.3	49.7	80.6	57.6	77.7
hsa-miR-99b*	117.1	144.9	114.7	165.4	106.5	136.9	155.9	154.4
hsa-miR-766	152.2	180.4	235.8	211.7	127.9	195.5	111.2	157.5
hsa-miR-185*	212.8	253.3	195.8	294.8	220.0	249.8	232.0	255.1
hsa-miR-630	113.4	119.3	102.0	134.8	82.5	127.5	110.7	117.8
hsa-miR-21*	184.9	224.2	238.6	249.5	176.2	251.8	136.9	183.2
hsa-miR-125b-1*	75.5	80.1	71.2	96.7	61.2	88.5	81.8	94.3
hsa-miR-505*	139.9	153.5	125.4	182.4	109.8	158.0	136.9	155.4
hsa-miR-382	84.2	98.0	74.7	110.3	66.7	90.3	87.0	97.0
hsa-miR-617	119.1	120.9	95.2	135.6	81.1	123.5	105.6	118.6
miRPlus_42521	413.6	473.8	508.0	493.5	237.6	429.2	417.4	533.8
hsa-miR-934	139.3	160.0	124.5	169.5	90.6	148.9	128.5	143.7
hsa-miR-488	62.4	68.5	60.1	81.3	46.0	76.3	63.3	71.8
hsa-miR-302d*	408.1	451.2	428.7	547.9	307.8	500.2	293.9	367.0
hsa-miR-423-5p	921.6	1104.3	954.3	1368.0	892.4	1389.3	904.4	978.3
hsa-miR-891a	133.4	167.0	129.6	188.3	116.9	157.9	142.3	173.6
hsa-miR-124	45.7	65.3	51.5	78.8	51.9	73.3	63.2	58.7
hsa-miR-634	438.3	514.0	508.3	625.4	335.6	537.6	417.7	529.3
hsa-miR-596	58.2	66.9	86.5	76.6	39.6	70.9	47.1	72.7
hsa-miR-877	128.2	154.6	108.4	171.5	113.9	146.9	143.1	166.7
hsa-miR-642	791.7	820.1	767.9	833.2	363.9	718.2	551.2	804.8
hsa-miR-206	65.2	74.9	52.8	81.1	42.2	66.8	66.3	78.6
hsa-miR-185	1048.3	1125.9	765.4	1310.1	797.4	1311.5	950.8	1067.7
hsa-miR-516b	97.0	112.6	89.9	137.6	71.3	114.2	109.4	124.3
hsa-miR-184	321.3	410.0	347.0	479.8	217.8	386.0	451.6	471.2
hsa-miR-187*	132.4	151.7	123.3	169.9	80.1	150.4	134.6	160.9
hsa-miR-155	785.9	831.1	657.0	1055.5	541.3	1000.0	789.7	894.0
hsa-miR-638	237.5	318.7	376.0	303.8	132.3	275.1	204.3	330.5
hsa-miR-526b	112.7	120.5	92.1	144.6	66.2	131.3	112.1	126.6
hsa-miR-671-5p	69.2	96.8	86.7	110.1	64.3	101.4	72.3	98.3
hsa-miR-214	132.8	164.8	131.7	197.7	93.2	173.8	148.9	167.9
hsa-miR-299-3p	93.4	105.8	71.7	126.9	57.0	100.4	108.9	119.3

hsa-miR-525-5p	157.7	189.6	180.0	205.5	94.4	188.8	132.9	183.7
hsa-miR-409-5p	119.8	119.6	93.4	141.9	50.9	124.0	113.9	128.5
hsa-miR-513a-5p	1101.8	1077.4	840.3	1234.2	482.1	1213.3	1062.0	1148.1
hsa-miR-198	94.7	128.4	94.9	143.8	75.1	129.6	117.0	132.3
hsa-miR-498	159.5	200.2	166.5	211.9	105.0	202.7	140.6	184.4
hsa-miR-519e*	184.4	219.8	158.3	253.3	128.0	223.6	160.3	204.4
hsa-miR-509-5p	183.1	212.1	153.2	248.9	120.2	230.2	158.1	191.1
hsa-miR-326	121.6	161.5	92.7	188.1	128.4	156.8	111.8	150.8
hsa-miR-510	106.9	130.8	99.0	157.9	77.6	145.8	112.5	134.9
hsa-miR-574-5p	871.9	947.6	629.4	1075.6	429.3	976.3	697.3	767.4
hsa-miR-483-5p	126.7	160.3	95.4	169.9	82.8	150.4	141.4	167.1
hsa-miR-659	93.1	117.4	82.4	133.8	65.0	121.1	93.7	112.5
hsa-miR-422a	220.1	244.3	183.1	279.2	108.9	246.4	204.8	242.0
hsa-miR-503	949.4	1157.6	914.8	1363.8	617.6	1368.3	903.9	1053.0
hsa-miR-200b*	84.6	108.3	94.8	119.1	52.2	115.7	77.8	108.6
miRPlus_17955	135.4	176.8	143.7	195.9	80.9	162.9	131.2	177.5
hsa-miR-490-3p	83.2	94.8	65.4	110.8	46.9	105.5	72.5	86.0
miRPlus_42526	2404.9	2905.1	2450.6	3401.2	1299.5	4122.5	2844.6	2708.5
hsa-miR-25*	164.1	230.1	167.3	246.0	143.7	249.3	213.3	300.5
hsa-miR-183*	141.1	199.9	173.7	226.5	114.0	202.9	128.8	188.3
hsa-miR-193b*	76.4	109.0	75.4	130.9	63.8	117.0	93.7	110.7
hsa-miR-551a	99.5	132.3	87.7	156.5	68.7	138.6	157.8	179.1
hsa-miR-620	3113.9	2511.6	1524.4	2929.5	701.6	2552.8	2579.7	2668.5
hsa-miR-937	65.2	95.9	73.3	116.0	60.4	100.6	80.7	111.0
hsa-miR-516a-5p	261.9	358.6	208.3	416.7	207.5	342.2	272.1	350.1
hsa-miR-492	67.3	120.6	121.6	134.9	59.0	118.6	95.9	137.2
hsa-miR-519c-5p/hsa-miR-519b-5p/hsa-miR-523*/hsa-miR-518e*/hsa-miR-522*/hsa-miR-519a*	146.4	201.5	167.3	233.3	89.5	199.0	142.3	199.2
hsa-miR-628-3p	746.8	1007.9	910.8	1258.9	542.2	1344.5	631.4	907.1
hsa-miR-551b*	133.9	153.1	104.3	190.6	71.4	167.2	120.9	163.3
hsa-miR-933	3634.1	3547.4	2678.7	4632.9	1463.8	5010.2	3012.1	3356.8
miRPlus_42793	336.9	467.5	282.2	532.7	222.2	457.8	341.2	426.8
hsa-miR-526b*	50.4	73.7	51.1	89.7	41.7	85.2	54.2	78.8
hsa-miR-765	1282.7	1379.0	857.3	1715.0	524.5	1657.0	1215.1	1353.7

hsa-miR-663	52.8	81.6	70.3	87.1	30.6	86.4	65.7	87.2
hsa-miR-520d-5p	583.4	755.5	530.1	937.4	399.2	852.0	450.4	656.5
hsa-miR-939	777.5	974.4	689.2	1276.5	455.0	1208.1	674.6	839.2
miRPlus_42745	866.1	1097.5	1503.3	1381.2	420.3	1478.9	628.9	1164.2
hsa-miR-518a-5p/hsa-miR-527	276.3	346.9	215.9	402.8	151.4	341.2	230.5	336.8
hsa-miR-193a-5p	1665.2	1972.1	2142.9	2399.1	706.4	2531.2	1131.7	1867.6
hsa-miR-10a*	46.7	63.4	42.2	77.1	34.9	74.6	50.1	75.5
hsa-miR-923	14867.2	9951.6	7596.3	15887.3	2582.3	25041.1	15427.1	9207.8
miRPlus_42487	9879.3	10915.0	10428.4	12840.7	3738.7	18320.5	7707.9	9438.5
hsa-miR-583	454.6	612.4	338.5	747.2	304.9	681.7	509.8	641.7
miRPlus_27560	197.1	387.0	235.6	421.3	188.2	364.0	340.3	429.1
hsa-miR-542-5p	73.4	102.4	52.4	123.9	49.6	103.7	100.1	124.3
hsa-miR-149*	355.5	580.0	419.7	720.4	320.7	722.3	327.1	471.5
hsa-miR-518c*	732.4	902.3	524.7	1101.5	356.9	1037.6	674.2	814.3
hsa-miR-874	373.6	486.3	645.2	603.1	146.3	510.0	205.0	444.0
miRPlus_17869	1344.9	1771.9	1055.8	2369.5	830.9	2335.7	1592.1	1871.4
hsa-miR-32*	2029.8	2288.9	1313.9	2731.1	743.0	2966.4	1713.9	1869.9
miRPlus_28431	1701.9	2410.6	2121.6	2748.9	856.9	3101.5	1534.7	2381.2
hsa-miR-298	1029.4	926.6	530.1	1154.4	208.4	924.5	788.0	954.7
hsa-miR-675	112.3	203.2	150.5	230.2	93.7	201.1	121.7	219.0
hsa-miR-7-2*	68.9	101.0	57.1	120.8	40.9	102.4	74.9	107.6
hsa-miR-658	784.0	1054.2	613.9	1284.0	421.0	1256.9	761.1	1000.9
hsa-miR-30b*	1157.9	1738.8	1120.4	2019.1	569.7	2009.7	1216.6	1605.5
hsa-miR-557	66.3	96.7	81.6	102.6	31.2	109.4	54.5	99.1
hsa-miR-30c-1*	82.4	128.9	61.0	147.7	52.9	127.5	87.9	141.2
hsa-miR-665	1263.5	1758.9	1092.6	2229.2	537.9	2228.1	1157.6	1564.9
hsa-miR-30c-2*	758.1	1091.0	504.0	1463.0	438.5	1475.3	784.9	1020.1
hsa-miR-602	435.2	646.0	411.2	837.2	146.9	656.9	496.3	746.9
hsa-miR-551b	1304.0	1860.3	846.3	2331.3	654.2	2320.5	1148.5	1599.3
hsa-miR-640	65.9	116.1	56.7	137.8	40.1	117.5	74.5	123.4
hsa-miR-631	47.0	81.8	37.8	99.0	28.6	88.2	49.9	81.0
hsa-miR-552	142.0	193.1	84.0	248.7	46.4	211.0	171.5	215.2
hsa-miR-371-5p	2970.7	4341.8	4762.3	6237.0	1203.9	7904.4	1898.2	4030.6
hsa-miR-921	1373.2	1993.7	989.8	2697.9	552.8	2683.2	1485.2	1949.4
hsa-miR-381	412.9	558.0	226.2	668.5	103.4	534.4	390.8	534.0
hsa-miR-494	3419.3	4205.0	1752.0	5739.3	654.0	5342.7	3177.8	4387.9
miRPlus_17952	9573.6	23388.1	3424.2	32527.3	1020.0	40380.7	9359.0	20890.0

Supplemental Table 5. Sequences for anti-miRs

Name	Sequence
hsa-miR-126	5'-CGCATTATTACTCACGGTACGA-3'
hsa-miR-130a	5'-ATGCCCTTTTAACATTGCACTG-3'

Supplemental Table 6. Sequences for miR-mimics

Name	Sequence
hsa-miR-126	5'-UCGUACCGUGAGUAAUAAUGCG-3'
hsa-miR-130a	5'- CAGUGCAAUGUUAAAAGGGCAU-3'

Supplemental Table 7. Sequences of primers for quantitative real time PCR

Name	Forward sequence	Reverse sequence
Spred1	5'-CGGCGACTTCTGACAACGAT-3'	5'-TTGAGTCATCTCGGGTCATCAC-3'
HOXA5	5'-TCTCGTTGCCCTAATTCATCTTT-3'	5'-CATTCAGGACAAAGAGATGAACAGAA-3'
EGFL7	5'–CAGACGGTACACTCTGTGTGC–3'	5'–CAGCACCAGCTGCAGCTTCT–3'
hL28	5'–GCATCTGCAATGGATGGT–3'	5'-CCTTTCTCCTGGCCCATACAC–3'
TBP	5'-TGCACAGGAGCCAAGAGTAA-3'	5'-CACATCACAGCTCCCCACCA-3'
GAPDH	5'– GAAGGTGAAGGTCGGAGTC –3'	5'– GAAGATGGTGATGGGATTTC –3'

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